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The Absorption and Utilization of Direct, Diffuse and Low Angle Light by Plant Leaves

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THE ABSORPTION AND UTILIZATION OF DIRECT, DIFFUSE AND LOW ANGLE LIGHT BY PLANT LEAVES

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ABSTRACT

The light environment of plants is extremely complex and questions relating to how direct, diffuse, or low-angle light affect plants at the leaf-level have remained largely unanswered. Global-change scenarios suggest a trend of increasing diffuse light due to expected increases in cloud cover and atmospheric water vapor concentrations. Here we present three different examples where changes in the directional quality of light affect leaf-level processes. First, some understory plants have well-developed lens-shaped epidermal cells, which have been shown to focus collimated light, but their optical function under diffuse light has been largely speculative. To assess the role of epidermal cell shape in capturing direct vs. diffuse light, we measured leaf reflectance and transmittance with an integrating sphere system using leaves with flat and lens-shaped epidermal cells. Regardless of epidermal cell shape, direct light was absorbed more than diffuse light in all species studied by approximately 2–3%. These data suggest that lens-shaped epidermal cells do not aid the capture of diffuse light, and palisade and mesophyll cell anatomy and leaf thickness appear to have more influence in the capture and absorption of light than does epidermal cell shape. Second, community-level productivity has been shown to increase under diffuse light conditions and has been attributed to more uniform distribution of light within the forest canopy. Leaf-level responses to the directional quality of light, however, are unknown. Here we show that leaf-level photosynthesis in sun leaves of both C_3 and C_4 plants can be 10–15% higher under direct light compared to equivalent absorbed irradiances of diffuse light, while shade-adapted leaves showed no preference for direct or diffuse light at any irradiance. Sun leaves with multiple palisade layers may be adapted to better utilize direct than diffuse light, while shade leaf structure does not appear to discriminate light based on its directionality. Thus, it appears that leaf-level and canopy-level photosynthetic processes react differently to the directionality of light, and previously observed increases in canopy-level photosynthesis occur even though leaf-level photosynthesis decreases under diffuse light. Third, we tested how changes in the directional quality of light affect the penetration of light at the leaf-level. Using chlorophyll fluorescence imaging we were able to determine that low-angle and diffuse light do not penetrate as deeply into leaves as direct light. Upon entering the leaf, diffuse light appears to scatter and remain in the upper tissue layers, while direct light penetrates through more leaf tissue. Absorption of diffuse light is reduced compared to direct light, with the greatest differences in absorption occurring near the interface of the palisade and spongy mesophyll tissue. Changes in the directional quality of light can therefore alter the absorption of light at the leaf-level, and a shift in the absorption profile could potentially decrease light utilization, potentially contributing to the leaf-level photosynthetic differences observed. Overall, it is now clear that plants are much more sensitive to the directional quality of light than we once believed. Also, the directional quality of light has different effects when scaling from the leaf to the landscape, and models of both leaf-level and community-level photosynthesis should be revised to account for these new findings.

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CHAPTER 1: LITERATURE REVIEW

The quality and quantity of light reaching photosynthetic plant tissue have long been recognized as critically important factors affecting the assimilation of CO_2 by plants. Until recently, however, the directional quality of light incident at the leaf-level has been overlooked as a potential source of variability in the utilization of light. Community-level research has shown that plant canopies benefit from greater proportions of diffuse light, yet the effects of direct, diffuse, and low-angle light are virtually unknown at the leaf-level. This body of research aims to determine how plants respond to changes in the directional quality of light, and whether absorption and utilization are affected by that directional change. Light arriving as collimated, diffuse, and low-angle light incident at the leaf surface were examined to determine how naturally occurring variation in the light environment of plants might affect photosynthesis.

Leaf Form & Photosynthesis

Some of the greatest differences in intraspecific morphological plasticity are often observed at the extremes of high and low light intensity. Adaptation to the variability within the light gradient between those two extremes is a critical factor in the success of land plants. Light intensity from the top to the bottom of a plant canopy can decrease by several orders of magnitude simply due to the absorption and reflectance of light by leaves (Larcher 1995). Across this extreme light gradient plants employ a variety of structural and biochemical adaptations to efficiently utilize light in a wide range of environments.

Sun vs. Shade Leaf Morphology

Leaf morphology is driven by the search for, interception of, and maximum utilization of light. Sun and shade leaves, as their names imply, exist in drastically different light environments (Niinemets 2007). Low and high irradiance can both be limiting to photosynthesis, and plants are able to adapt to light environments through fine-tuning their morphology and biochemistry.

Sun-adapted leaves will spend the majority of their "lifetime" in areas of high solar irradiance. Eventually other leaves or branches of the same plant, or other plants, may begin to shade the leaf, and this often induces senescence of the leaf. The high light environment of a sun leaf includes not only radiation in the photosynthetically active range (400-700nm), but also in the ultraviolet (UV) and far-red ranges. Construction costs for sun and shade leaves of plants vary, with shade leaves often being 1-5% lower in cost than thicker, more nitrogen-rich sun leaves (Poorter et al. 2006). However, the disparity is great between the number of days required to replenish the resources used in leaf production in sun and shade leaves, ranging from 2-3 times longer for shade leaves, primarily due to the lower irradiance present in the understory or shaded environments. Despite this imbalance, given the integrated irradiance present in most shaded environments, construction costs are typically reclaimed and production of shade leaves is cost-effective (Niinemets 1999; Poorter et al. 2006).

Sun adapted leaves typically have a smaller leaf area and greater leaf thickness and mass per unit area than their shade counterparts (Larcher 1995). Along with being smaller (displaying a lower leaf area to the sun) they are often more deeply lobed, allowing leaves to remain closer to ambient air temperature (Vogel 1970; Schuepp,

1993). Lobed leaves may also allow for increased air turbulence, and thereby decrease the boundary air layer that limits gas exchange capabilities (Schuepp 1993). These characteristics are the result of cooling strategies by plants. High light environments allow for more light interception and higher photosynthetic rates, but also generate a considerable amount of heat, both from exposure to high intensity sunlight and the biochemical reactions within the leaf. To avoid excessive heat, plants attempt to minimize the boundary air layer that surrounds the leaf. The majority of stomata occur on the underside of a leaf and this is where the majority of gas exchange occurs, allowing CO_2 into the leaf for photosynthesis, and water to leave via transpiration which creates evaporative cooling. Some leaves have stomata on both adaxial and abaxial surfaces, aiding the diffusion of CO_2 to the sites of photosynthetic activity (Parkhust & Mott 1990).

Reduction of the boundary air layer is important for the rapid diffusion of CO_2, O_2, and water vapor. If the boundary air layer thickness is saturated with water vapor, the diffusion gradient that exists between the intercellular air space of the leaf and the outside environment is reduced, and the leaf will not be able to effectively cool itself (Grace & Wilson 1975; Young 1985). The thickness of the boundary air layer is proportional to the leaf size, shape of the leaf, and the direction and speed of the air movement (Vesala 1998). By developing a thicker leaf, with a smaller total leaf area, the boundary air layer of a sun leaf will be reduced. Lobed leaves will also cause the leaf to flutter more in the presence of even low wind velocities, thus increasing the amount of gas exchange the leaf can accomplish (Stokes et al. 2006). This fluttering effect also occurs in strap-shaped leaves and has important consequences for graminoid plants. Parlange and Waggoner

(1972) found that there were differences of up to 15° C in leaf temperature between the leading and trailing edge of non-transpiring *Phragmites communis* leaves, and the resulting turbulence caused decreases in the boundary layer resistance by about 40% compared to laminar breeze with leaf flutter/flapping inhibited.

At the whole-leaf level, shade leaves differ in a variety of ways from sun leaves. Shade leaves receive solar radiation that is filtered by other leaves and hence is of different spectral quality and quantity. As light passes through the upper canopy layers, it is scattered and becomes diffuse by the time it reaches the ground. Diffuse light reaching the ground is depleted in the red and blue wavelengths due to absorption by upper leaf layers. Thus, shade leaves are required to utilize diffuse light, primarily composed of green light not absorbed by other plants. Shade leaves are usually thinner and display a greater total leaf area relative to leaves growing in high light. These leaves can have a larger area because the solar radiation that they receive is reduced, and they do not experience the potentially excessive heat load that sun leaves do. Low wind velocities in the understory, however, reduce convection and gas exchange for large leaves. Because boundary air layer thickness increases with lower wind velocity, the large shade leaf will have more difficulty in lowering leaf temperature via transpiration and convection. One solution to this problem is wilting. By changing the orientation of the leaf from horizontal to vertical, or some leaf angle in between, the warm air of the boundary air layer can simply rise and will not be trapped underneath the large flat surface. Changes in leaf angle via wilting are employed by both sun and shade leaves, and steeper leaf angles can result in lower midday heating and increased water use efficiency (King 1997). Steep leaf angles, however, have the potential to decrease the total amount of

light intercepted by the leaf at midday, but when the solar angle is lower in the afternoon, light interception increases. Therefore, plants with leaves at steep angles avoid midday extremes in light intensity and heat, while still maximizing total carbon gain (Falster & Westoby 2003).

The internal anatomy of sun and shade leaves also differs considerably. Sun leaves will typically have a thick epidermis, a thick multi-layered palisade, large spongy mesophyll cells, and relatively small intercellular air spaces (Terashima et al. 2006). The thick epidermis may partially reduce the effects of photodamage from UV radiation. In conifer species, the chemical composition of epicuticular waxes has been shown to change in response to UV radiation, typically with the increase of UV radiation absorbing compounds such as flavonoids (Gordon et al. 1998). Production of epicuticular waxes for protection from photodamage, however, can come at the expense of higher leaf temperatures and decreased diffusion of gases. This is due to the insulating effects of the waxes and increases in resistance to diffusion at the stomata (Mohammadian et al. 2006). The epidermis and other leaf tissues will often contain anthocyanins and xanthophylls that act to absorb excess light and dissipate heat to prevent photodamage. The thick palisade layer may channel light deeper into thicker sun leaves, allowing more uniform distribution to the photosynthetic tissues. Sun leaves generally have a higher number of chloroplasts per leaf area than shade leaves, and they are often smaller than chloroplasts of shade leaves. In high light, these smaller chloroplasts will be oriented along the periclinal walls, closest to the source of CO_2 rather than in the center of the cells. Smaller chloroplasts decrease the diffusion distances within the aqueous phase within cells, as CO_2 drawdown is proportional to the diffusion rate across the various resistances

(stomata, cell wall, plasma membrane, etc.) between the site of carboxylation and the outside environment (Terashima et al. 2006). Because the diffusion rate of CO_2 within water is approximately 10,000 times slower than in air, the size and placement of chloroplasts is critical for supplying CO_2 to meet the demand of cells that are photosynthetically active (Weast 1979; Nobel 2005). Sun leaves typically have a higher density of stomata on the abaxial side of the leaf, and some sun leaves will develop stomata on the adaxial side to increase gas exchange and evaporative cooling. These qualities allow sun leaves to have a higher photosynthetic rates, stomatal conductance, light saturation point, and higher transpiration rates. The increased thickness of sun leaves is a compromise between light harvesting capability and construction costs, as thicker sun leaves require more energy and nutrients to produce and maintain.

In contrast, shade leaves have thinner epidermal, palisade, and mesophyll layers. The intercellular air spaces are typically larger in volume, which may aid in scattering light throughout the leaf. Specialized epidermal lens cells can focus direct light, and increase light intensity several fold (Vogelmann 1996). This light gathering solution is one of the ways shade plants efficiently utilize the limited amount of solar radiation they receive.

Leaf optical properties have been shown to be quite different between sun and shade leaves (Lee et al. 1979; Lee & Graham 1986; Lee et al. 1990). Extreme-shade plants more efficiently absorb light across the spectrum of photosynthetically active radiation (400-700 nm) due to lower reflectance and higher absorptance of incident light. Until recently, however, measurements of reflectance, transmittance and absorptance of light for plant leaves were only possible under direct or collimated light conditions,

where light arrives perpendicularly to the leaf surface. Technical limitations prevented the measurement of how diffuse light is absorbed by leaves. The light environment of the understory is complex, and can be composed of both direct and diffuse light. Therefore, determining whether plants absorb direct and diffuse light equally is important for the basic understanding of the effects of light environment on plants and how they adapt photosynthetically.

The leaf properties described above are typical for broadleaf species of both shrub and tree life forms. Some evergreen species will also exhibit similar morphological changes when growing in high light or low light environments. For example, subalpine fir (*Abies lasiocarpa*) has high phenotypic plasticity in response to their light environment. Under high light, shoots will develop needles that are tightly packed and round (Smith et al. 2003). However in the understory the lower branches of the same tree will have needles that are arranged horizontally and have a laminar shape.

Overall, plant phenology will also determine the light gathering properties of different plant forms. Deciduous trees when growing alone in a field with no neighbors will often develop a straight trunk and a globe shaped canopy or one that tapers to the top (Horn 1971). With no light competition by neighbors, trees can be limited by abiotic factors such as water, nutrient availability, and photoinhibitory effects of high light. The geometry of crown shapes, along with the density of leaves will determine leaf display on the inner regions of the tree closest to the main trunk. Conifers often have fewer needles on the interior of their crowns because the dense packing of needles at the ends of the branches absorb almost all of the available light for photosynthesis. When needles become shaded and do not produce enough photosynthate, they are shed so resources can

be translocated to the outer growing shoots. This situation also occurs in deciduous trees, but the more widely spaced leaf and branch display, as well as larger flat surface area exposed to sunlight, causes a significant amount of light to be reflected off the outer leaves to the inner portions of the crown, thus supporting a larger number of shade type leaves closer to the trunk (Larcher 1995).

Leaves also respond anatomically when they develop under high or low light. Sun leaves typically develop longer palisade cells, and they often have multiple palisade layers on the adaxial side of the leaf. Under very high light environments, leaves can also develop palisade layers on both the adaxial and abaxial sides. These leaves are often displayed vertically, so both sides of the leaf receive as much light as possible. The palisade cells usually have very few chloroplasts, and the majority of them reside in the spongy mesophyll tissue (Terashima & Hikosaka 1995; Lambers et al. 1998). Elongate palisade cells are thought to help channel light into the chloroplast-rich spongy mesophyll layer, where the light is scattered, thereby increasing the path length and ultimately light absorption (Vogelmann & Martin 1993; Vogelmann & Evans 2002).

Because intercellular resistances limit CO_2 diffusion through leaves, CO_2 concentrations are lower near the leaf's adaxial surface. In very high light environments, some plants with thick leaves develop stomata on the both top and bottom leaf surfaces. In addition to allowing CO_2 to diffuse directly into the palisade, these stomata also allow for additional evaporative heat loss via transpiration.

Internally, intercellular reflectance of light via scattering has been shown to be a significant contributor to photosynthesis in shade leaves (DeLucia et al. 1996), increasing absorptance by 25-30% in some species when compared to leaves treated with vacuum

infiltration that eliminates intercellular air space. The increased amount of intercellular air space in shade leaves is likely to be an adaptation to low light environments where utilization of all available light is critical for growth. This internal scattering and trapping of light within the leaf may give shade plants the ability to remain closer to the photosynthetic light compensation point that might otherwise not be achieved.

Various studies have shown that maximum carbon fixation does not actually occur in the top layers of leaves where light is most intense, but rather deep in the palisade tissue and in the spongy mesophyll (Terashima 1989; Cui et al. 1991; Nishio et al. 1993). While chlorophyll (primarily chlorophyll *a*) is responsible for the pattern of light absorption profile within leaves, the distribution of Rubisco distribution is strongly correlated to the CO_2 fixation capacity (Nishio et al. 1993; Nishio 2000).

Photosynthetic response to light intensity also varies between sun and shade leaves. The shape of light response curves for plants are asymptotic, with a linear response to light at low intensities, followed by saturating high light where little carbon assimilation is gained per unit of increasing light. Sun leaf photosynthesis saturates at higher irradiances, but the quantum yield is generally the same for sun and shade leaves (Lambers et al. 1998).

ADAPTATIONS TO THE LIGHT ENVIRONMENT

Plants growing in extreme environments offer a unique opportunity to observe the extent to which plant morphology can be stretched to allow successful growth and reproduction. Desert plants exemplify the extent to which plants can adapt to hot, arid environments. Deserts are extremely dry and do not support plant forms that depend on

large amounts of water for transpiration, and this is one of the main reasons broadleaf trees are so infrequent in desert environments (Larcher 1995). Often at low latitudes, the light environment of deserts is characterized by intense sunlight coupled with highly reflective sandy soils. Sand reflects a large amount of light, causing the ground of the desert to have an exceptionally high albedo (Campbell & Norman 1998). In general there is excess light in the desert for plants, and they have had to adapt to high light intensity and heat. Desert plant species will often have specialized epidermal structures to prevent photodamage from the intense solar radiation. Thick epicuticular waxes are often present, which not only block UV radiation, but also prevent water loss through the epidermis (Björn 2002). Desert plants are also known for their specialized epidermal cells, in the form of trichomes, pubescence, and spines that effectively block the majority of the UV radiation yet transmit photosynthetically active radiation. Darling (1989) showed that the epidermis and hypodermis of saguaro reflects most UV radiation and transmits ~70% of light in the 400-700 nm range to the tissue below for photosynthesis.

Trichomes, epidermal hairs, and spines are highly reflective, and they minimize the amount of damaging solar radiation that the photosynthetic tissue actually receives. They also act as an insulating mechanism to hold a layer of air between them and the surface of the plant tissue. This insulation feature is necessary as fluctuations in diurnal desert temperatures are great. Flavonoids located in the trichomes of olive leaves have the ability to absorb as much as 60% of the incident UV radiation and the screening properties of these structures allow only 5-10% of the total UV radiation to reach the mesophyll (Larcher 1995). Plant cell nuclei will often be positioned below the vacuole

which also contains screening pigments, so that any damaging UV radiation must travel through this UV-screening organelle before encountering plant DNA.

Trichomes can also increase the water repellency of a leaf's surface, allowing for efficient gas exchange (Ishibashi & Terashima 1995). In low light environments they can also be used to position water droplets above the surface of the leaf. This will effectively act as a ball lens and focus light into the deeper tissue layers and increase light intensity below the water droplets. Light intensity has been shown to increase up to twenty times incident light levels below these water droplets (Brewer et al. 1991).

The evolution of Crassulacean acid metabolism (CAM) in cacti, epiphytes, and other plant groups has allowed for the uptake of CO_2 during the night when temperatures are cool, instead of during the day when evaporative water loss is potentially very high. The CO_2 is stored as malic acid in the vacuole overnight, and it is then transported to the cytosol and chloroplasts where it is then decarboxylated in the presence of light during the day (Black & Osmond 2003). There are a variety of CAM-C_3 and CAM-C_4 intermediaries, where plants operate under the metabolic pathway that is most suitable for their particular environmental conditions.

In a study of Western Australian plant communities, Smith et al. (1998) found that decreasing annual precipitation and increasing total daily sunlight were strongly correlated to the number of species exhibiting steep leaf angles and thicker mesophyll layers. The presence of multiple palisade layers and both adaxial and abaxial stomata were also strongly correlated with environments with low rainfall and high light. In sun adapted shrub species, Ishida et al. (2001), leaves oriented vertically were able to

photosynthesize at higher rates and recover from potential photodamage in high light when compared to similar species without vertical leaf orientation.

Plants growing in the alpine and at high latitudes exemplify the adaptive capabilities of plants in cold environments, where sunlight can also be extremely intense. Along with cold temperatures, plants must adapt to high winds, intense light, low nutrient availability, and frequent drought stress. Low temperature photoinhibition is one of the more serious problems for plants at high altitudes. Cold, often freezing, night temperatures during the growing season can be followed by intense morning sunlight. In this situation, light levels exceed the requirements for photosynthesis, and electrons cannot be efficiently transferred to carbon due to enzyme limitations and photodamage (Robakowski 2005). Broadleaf trees that grow at high altitudes, such as various *Rhododendron* species, have two distinct methods for minimizing low temperature photoinhibition. The first is leaf curling, and the second is the ability to change leaf orientation (Heckathorn & DeLucia 1991; Smith 2008). Curling of the outer edges of the leaves into a cigar shape is common in at least two *Rhododendron* species at high altitudes as well as other grasses and C_4 species. The curling effect allows for a certain degree of self-shading, leaving about half of the leaf surface exposed to the morning sun, while the other half of the leaf is protected. Leaves typically uncurl throughout the day, and curl up again at night. Leaves usually stay curled for most of the winter. These same species will also change the orientation of their leaves to the cold night sky (Smith 2008). Leaf angle is usually coupled with increasing steepness of leaf angle relative to sky exposure. Conifer species (such as *Abies lasiocarpa* and *Picea engelmanii*) will also

display their needles in a vertical manner in high light conditions, and in low light conditions needles are typically fewer per shoot they are displayed horizontally.

Vertically arranged needles minimize the amount of leaf area exposed to direct sunlight and the clustering of erect needles provides self-shading (Smith et al. 1998). Measuring the silhouette leaf area to total leaf area ratio (STAR) has shown that in several alpine tree species that total leaf area can be increased by packing more needles onto a shoot in a vertical arrangement (Carter & Smith 1985). Sun shoots typically had a lower STAR value than those of shade shoots due to the arrangement of needles on the shoot. Because short growing seasons limit the elongation of shoot structures on these trees, increasing the number of needles per shoot is beneficial to the plant, despite the self shading that might occur. Photosynthetic levels remain relatively constant over a variety of STAR values.

Low lying cushion plants form as rosettes in the alpine tundra, and have leaves that are smaller and more densely packed at high altitude than those at lower elevations. Germino and Smith (2000) found that in *Caltha leptosepala* and *Erythronium grandiflorum* (both alpine perennials) growing near snow banks, that experimental manipulation of sunlight (shading) and temperature (warming) increased the maximum efficiency of photosystem II the following day. Both species appear to be very capable of reaching maximum photosynthetic levels even after nights with frost and intense morning radiation. Yet shading the plants from intense morning sunlight only caused minimal improvements in afternoon photosynthetic rates. The photosynthetic rates of these alpine plants were compared to both crops and conifer trees, which both showed much less tolerance for frost nights followed by high morning sunlight. These two species, which

often emerge from snow banks and experience extremely high light levels have adapted their physiology to allow recovery from low temperature photoinhibition. While the morphology of these two species is considerably different, they both have a tolerance to low temperature and high light. In another study by Germino and Smith (1999), they showed that the vertical display of cotyledons and primary needles of conifer seedlings increased maximum photosynthesis. The short stature of seedlings allow them to absorb a large amount of heat during the day from solar radiation, and then minimize heat loss by staying as close to the ground as possible.

Smith et al. (1997) found that light and associated stress levels often determine plant form. For plants living in high light and low stress (water and nutrients abundant) plants often have large broad leaves that are displayed horizontally toward the sun. These leaves often track the sun's movements throughout the day in order to maximize sunlight exposure. Plants in high light and high stress, however, typically develop smaller leaves that are oriented vertically or away from the sun.

Plants with leaves displayed horizontally often have symmetrical leaf anatomy, with palisade tissue on both the adaxial and abaxial sides. In open environments this is believed to be an adaptation to maximize light utilization per unit leaf area (DeLucia et al. 1991). When illuminated from the adaxial or abaxial side of a vertically displayed leaf, photosynthetic rates are often identical. This is not the case with leaves that are displayed horizontally, and the difference in photosynthesis from adaxial and abaxial illumination can be greater than 30%. The asymmetry of horizontally displayed leaves is thought to be an adaptation to maximize daily integrated carbon gain while also maximizing nutrient use efficiency. This asymmetry or dorsiventral development is

particularly important in the differentiation of sun and shade leaf morphology (Smith 2008). The variation in sun and shade leaf morphology has presumably been an adaptation to balance the opposing internal gradients of light and CO_2 (Smith et al. 1997).

The palisade mesophyll acts as a light channeling structure, allowing light to penetrate deep into the leaf (Vogelmann 1993; Vogelmann et al. 1996). As light reaches the intercellular air space, the change in refractive index between the cells and the air causes refraction. This can change the path that light travels through the leaf and instead of passing directly through, it is often reflected back into the leaf. This internal reflectance increases the probability of light absorption by chloroplasts (Vogelmann et al. 1996; DeLucia et al. 1996). The percentage of intercellular air space to total leaf area is an extremely important component of leaf anatomy, as the air space provides the interface of gaseous CO_2 to the liquid phase within the mesophyll cells (Terashima et al. 2006). Efficient diffusion of CO_2 through the air space and adequate exposure of mesophyll cells to the air space will result in higher rates of photosynthesis, that would otherwise be limited by low CO_2 levels and reduced Rubisco activity (Slaton and Smith 2002).

STUDY OF LEAF OPTICAL PROPERTIES

The study of leaf optical properties is not new and over the past forty years progressive technological advancements have allowed for a better understanding of the transmittance, reflectance, and absorptance of light by leaves (Vogelmann 1993, Jacquemoud & Ustin 2001). Early work on the subject included the measurement of the

absorption spectra of the wide range of leaf pigments involved in the capture and utilization of light, including chlorophyll *a*, chlorophyll *b*, the carotenes, and the xanthophylls (Billings & Morris 1951; Gates 1965; Wooley 1971; Kiang et al. 2007).

The transmittance of light through and reflectance from leaf surfaces has been traditionally performed with a spectrophotometer, monochrometer, and integrating (Ulbricht) sphere setup (Rabideau et al. 1946). Although newer methods exist, they are based on the same principles (Knapp & Carter 1998; Kiang et al. 2007). This method allows for the measurement of the amount of light reflected from, transmitted through, and absorbed by a leaf. It does not provide information about how and where light is absorbed inside the leaf. Transmittance and reflectance data from leaves are important in a variety of scientific fields, including remote sensing, ecophysiology and ecology. Due to the unique spectral signal produced by plants, a variety of plant characteristics can be detected by satellites including water stress, nutrient status, and physiochemical stress (Carter 1993; Carter & Knapp 2001; Scotford & Miller 2004).

Determining where light is absorbed within the leaf and how photons travel through the leaf is critical in understanding how leaf structure influences light absorption for photosynthesis. Several techniques have been developed to gather these data, including using fiber optic microprobes and chlorophyll fluorescence imaging. The overwhelming majority of these studies have only been conducted under direct (collimated) light conditions (Gates et al. 1965; Woolley 1971; Gausman and Allen 1973; Lee et al. 1986, 1990; Knapp and Carter 1998; Carter and Knapp 2001; Carter & Spiering 2002). Only one study to our knowledge (Hume 2002) has attempted to measure the

reflectance of total diffuse light from a leaf surface. These diffuse reflectance values were based on radiance measurements of reflected light, and suggest a potential two-fold increase in absorptance compared to direct light.

Leaf Optics

Leaf optical properties are largely determined by two phenomena. The sieve effect results from the heterogeneous distribution of chlorophyll in leaves. Packaging light absorbing pigments in chloroplasts means that light can pass through a leaf without being absorbed, hence the reference to a sieve. Where chloroplasts are located throughout the leaf is much more important than the total quantity, as strategic placement of chloroplasts will maximize light utilization (Lambers et al. 1998; Smith et al. 2004). Cell size, shape and the intercellular air spaces lead to the second important phenomena, light scattering. As light passes through leaves it can be scattered by cell-air interfaces, thereby increasing the path of light within the leaf, increasing the likelihood of absorption (Vogelmann 1993). The scattering effect acts as a trap light in the leaf and establishes the light microenvironment within the leaf. This microenvironment is heavily influenced by leaf anatomy and the distribution of chlorophyll and photosynthetic enzymes.

Leaf reflectance decreases significantly when leaves are infiltrated with oil or water. It has been shown that complex, dorsiventral leaves have a greater ability to scatter light internally than less complex leaf types due to the increased number of cell-air interfaces (Gausman & Allen 1973). This reflectance is due to light traveling through hydrated cellular tissue with a refractive index of ~1.3-1.5 into the intercellular airspace with a refractive index of 1.0. The change in refractive index causes the light path to

change, thereby increasing the scattering of light within the leaf tissue. Filling the intercellular airspaces with a fluid eliminates the abrupt change in refractive index between the air and cells, which is the source for reflection of light. Such a treatment indicates that much of the reflectance from a leaf originates from inside the leaf tissue (Pearman 1966; Woolley 1971; Gausman & Allen 1973).

One study of 48 different species found only a weak relationship between leaf thickness and reflectance in the near infrared (NIR, 750-1350 nm). This suggests that variations in intercellular airspace between species may be more important in determining reflectance than leaf thickness at these wavelengths (Slaton et al. 2001; Hume 2003). Further, Baldini et al. (1997) suggest that leaf water content may be more critical in determining leaf transmittance and reflectance than leaf thickness. Slaton et al. (2001) did find, however, that NIR reflectance was highly correlated with the ratio of mesophyll area to total area, leaf bicoloration, and thicker leaf cuticles. In a similar study Knapp and Carter (1998) found a strong relationship between NIR reflectance and leaf thickness. This study also found that leaf thickness was the best predictor of internal light scattering and NIR reflectance. It appears as though habitat strongly influences leaf optical properties, due to the water and nutrient status of the local environment. Reflectance data in the NIR range of wavelengths are important to the remote sensing community as reflectance values are much higher in the NIR than in the visible range of the spectrum, allowing for a strong signal from plants to be perceived by satellites.

LEAF PIGMENTS RELEVANT TO LIGHT ABSORPTION

Leaves of higher plants utilize a suite of pigments to harness energy from the sun to fix CO_2 and store it in the form of carbohydrate. The pigments involved include chlorophyll *a* and *b*, the carotenoids, and xanthophylls, and typically about 85% of light within the 400-700 nm range are absorbed (Smith et al. 2004). Chlorophyll *a* absorbs light across photosynthetically active range (400-700 nm), with strong absorption peaks in the blue (450 nm) and red (680 nm) wavelengths. The remaining pigments aid in broadening the range of wavelengths that can be utilized for photosynthesis. There is, however, a weaker absorption in the green wavelengths (~550 nm), due to increased transmittance and decreased reflectance of these wavelengths by leaves in this region of the spectrum (Nishio 2000). Despite the strong reflectance of green wavelengths by leaves, green light plays a critical role in driving photosynthesis deep within the spongy mesophyll (Sun et al. 1998).

In higher plants different groups of pigments exist that are used for light capture, Photosystems I & II and the Light-Harvesting Complexes I & II. The reaction center pigments are composed of chlorophyll *a* and carotenes, while the light harvesting complexes act as antenna systems composed of chlorophyll *a* and *b*, and are often paired with xanthophylls (Smith et al. 2004). Photosystem I is composed of a chlorophyll dimer with a strong absorption peak near 700nm. It is composed of approximately 110 chlorophyll *a* molecules, as well as a small amount of chlorophyll *b* and other proteins that aid in positioning the whole complex in the thylakoid (Lambers et al. 1998).

Photosystem II has a strong absorption peak near 680nm with a 3:1 ratio of chlorophyll *a* to *b*, as well as associated pigments for thylakoid positioning. A significant portion of the chlorophyll in leaves is located in the light harvesting complexes. This chlorophyll traps light and directs that energy toward the reaction centers. Most of the chlorophyll *b* is associated with the light harvesting complexes and not in Photosystem I or II (Anderson & Beardall 1991).

Carotenoids, including the pigments involved in the xanthophyll cycle (violaxanthin, anteraxanthin and zeaxanthin), are utilized by plants in high light environments to dissipate light exceeding the requirements for photosynthesis as heat. Under high light, excess protons accumulate in the thylakoid lumen and the increased acidification acts as a feedback signal triggering the conversion of violaxanthin into zeaxanthin, which alters energy transfer within photosystems, dissipating the absorbed light energy as heat (Demmig et al. 1987; Lambers et al. 1998). Without dissipating this excess energy as heat or fluorescence, the absorbed energy and associated electron transport would convert oxygen into a variety of reactive oxygen species that have the potential to damage chloroplast pigments as well as surrounding membranes (Demmig-Adams & Adams 1992). The violaxanthin to zeaxanthin conversion is rapidly reversible in low light.

Anthocyanins, a by-product of the flavonoid pathway, have recently been given more recognition as important pigments in plant leaves (Gould 2004). They appear to play a functional role in maintaining antioxidant levels in both juvenile and senescing deciduous leaves. In both of these developmental stages the leaf is not fully functional and able to respond to stressful environmental changes, as the full suite of

photoprotective pigments have either not been developed or have been translocated and degraded (van den Berg & Perkins 2007).

Anthocyanins are often associated with particular developmental stages in leaves, such as growth flushes of new leaf material and in senescing leaves of deciduous trees (Gould 2004). Often, however, those pigments are present throughout the lifespan of the leaf, but the concentration of other pigments mask the presence of anthocyanins. Stored in the vacuole, anthocyanins absorb light most strongly within the 500-600 nm wavelengths, and also in the high-energy wavelengths near 400 nm (Gould 2004). There is also evidence that these pigments may act in a variety of ways as anti-herbivory compounds. Typically, however, anthocyanins are believed to play a major role in the prevention of the negative effects of photoinhibition, where they scavenge reactive oxygen species generated by the chloroplasts during periods of intense light exposure, as well as by absorbing excess light that would otherwise be channeled to chlorophyll (Gould et al. 2002). Anthocyanins also act along with other flavonoids to block UV radiation that has the potential damage DNA.

MEASURING LIGHT ABSORPTION WITHIN LEAVES

Measuring the light environment within plant tissues has been attempted by a variety of methods. Two different strategies have emerged, each with their own strengths and weaknesses. Most recently absorption profiles within several species of leaves have been documented, producing robust datasets (Evans & Vogelmann 2003).

Fiber optic micro-probe measurements

Early experiments were done by Vogelmann & Björn (1984) using fiber optic microprobes to measure the internal light environment within a variety of plants. This technique offered a unique method for measuring the optical properties of the different cell layers within leaves (Vogelmann et al. 1991). Using a modified optical fiber (200 μm diameter) with a tip reduced to 20-70 μm in diameter they were able to insert the probe into leaf tissue and examine light being transmitted through the leaf. Observations included the quantity and spectral quality of that light. Just below the epidermis, light levels were approximately 1.2 times greater than incident light at the surface due presumably to internal light scattering (Vogelmann 1989; Vogelmann et al. 1988, 1989). Also, while the light environment within one particular portion of leaf tissue may be similar to those with similar anatomy, other regions that contain vascular tissue, which are devoid of chloroplasts, may be significantly different. The role of light channeling through the vascular tissue needs to be examined, as well as bundle sheath tissue in C_4 plants. Bornman et al. (1991) also used the fiber optic microprobe technique to measure chlorophyll fluorescence within leaves, finding maximum fluorescence near the boundary of the palisade and spongy mesophyll.

Some efforts have been made to measure light gradients in leaves by observing the phytochrome response in etiolated leaves as an *in vivo* proxy for light penetration. This technique allows for the measurement of light attenuation in leaves that lack chlorophyll (Seyfried & Schäfer 1985; Kunzelmann et al. 1988; Vogelmann 1989). These studies showed that light gradients had different shapes, depending on the wavelength of light that illuminated the etiolated leaf. The different shapes of the light

profiles matched well with predicted profiles from mathematical calculations (Vogelmann 1989).

Errors inherent in the estimation of the angular distribution of light measured with the fiber optic microprobe technique were resolved via a series of mathematical corrections by Richter & Fukshansky (1996), thereby accounting for the inherent limitations of the field of view of the microprobes.

Chlorophyll fluorescence as a proxy for light absorption

Assuming that the quantum yield for chlorophyll fluorescence is similar throughout the leaf, it is possible to use chlorophyll fluorescence as a proxy for light absorption (Takahashi et al. 1994; Koizumi et al. 1998; Vogelmann & Han 2000; Vogelmann & Evans 2002; Evans & Vogelmann 2006). The experimental set-up for this measurement involves irradiating a leaf sample with monochromatic collimated light that is directed to the leaf surface. This sample is mounted on the stage of a microscope and a cross-sectional view is observed through the microscope. Monochromatic light that enters the adaxial or abaxial surface, travels into the leaf and stimulates chlorophyll fluorescence, which escapes from the cross-sectional surface and is observed through the microscope. Chlorophyll fluorescence is recorded through digital images of the cross-sectional view, and fluorescence profiles are extracted from these images through image processing (Takahashi et al. 1994; Koizumi 1998; Vogelmann & Han 2000).

This method has shown that absorption profiles within leaves are relatively steep, and that most of the light is absorbed within the first ~15-20% of leaf tissue. Leaf optical

properties determine the shape of the light absorption profile (Cui et al. 1991; Vogelmann et al. 1989). Once light enters the leaf a light intensity gradient is established, where light is attenuated by either absorption or scattering. This causes the shape of the profile to be gradual or steep after the initial absorption peak (Vogelmann 1989). The penetration of light into leaf tissue is also highly dependent on wavelength. Red and blue wavelengths are absorbed strongly in the upper layers of leaf tissue, while green light penetrates much deeper into leaves and drives a considerable portion of the photosynthesis taking place in the spongy mesophyll tissue (Sun et al. 1998).

Due in part to the opposing gradients of light and CO_2 within most leaves, the highest photosynthetic rates do not occur at the adaxial surface where light intensity is highest. Instead, photosynthetic rates are greatest in the middle and lower palisade tissue, where there is a balance between light intensity, CO_2 concentration, Rubisco, and other photosynthetic enzymes (Fig. 1.1) (Nishio et al. 1993; Evans 1995; Sun et al. 1998; Sun & Nishio 2001; Evans & Vogelmann 2003). There has been debate over how well light absorption profiles and CO_2 fixation capacity overlap (Nishio 2000; Evans & Vogelmann 2003). However, it appears as though carbon fixation profiles do agree with profiles of light absorption as predicted by Beer-Lambert law and Rubisco distributions (Evans 1995).

One limitation to the chlorophyll fluorescence imaging method is that the leaf must be cut so that the transverse section is exposed to the microscope objective. This potentially alters the optical properties of light entering the leaf. By isolating chlorophyll fluorescence near the strongly absorbed ~680 nm wavelengths, the method is more reliable. Fluorescence at longer wavelengths can scatter and travel through the leaf, thus

spatially degrading the data (Vogelmann & Evans 2002; Evans & Vogelmann 2003). Also, cells that are free of chlorophyll can be illuminated by fluorescence from surrounding cells, giving an erroneous impression of high chlorophyll content and light absorption. This artifact appears commonly in the epidermis of leaves and needs to be taken into account when constructing absorption profiles within the mesophyll from chlorophyll fluorescence images by excluding epidermal layers from the analysis.

DIRECT VS. DIFFUSE LIGHT

Canopy Penetration

There is wide variation in the directional quality of light in the natural environment. On a clear, cloudless day sunlight arrives in beams at the earth's surface, composed of approximately 85% direct light and 15% diffuse light that is scattered by the atmosphere (Bird & Riodan 1986). On a cloudy day, nearly 100% of the incoming light is diffuse, as clouds, haze, or fog scatter the light before it reaches the earth's surface. It is not uncommon for clouds to pass over plant communities throughout the day, and those clouds can change the directional quality of light for minutes or hours. Plants must be able to utilize light arriving in direct beams or as diffuse, scattered light.

Clouds are often the medium that diffuses light over forests, causing a shift in the spectral quality of the light toward longer wavelengths (Dye 2005). Plant canopies will also scatter light and change the spectral composition of light arriving at the forest floor. Leaf litter and undergrowth acts as yet another filtering and diffusing layer capable of inhibiting seed germination (Vazquez-Yanes et al. 1990). Light diffusion by leaf litter

will lead to a shift toward longer wavelengths, thereby altering the red: far-red ratio. This ratio is critical to signaling involved in germination.

Diffusion of light by cloud immersion has been shown to reduce the variability of light intensity within an understory environment. The scattered light is more evenly distributed throughout the understory and light intensity generally increases. On clear, cloudless days much of the light is absorbed by the upper canopy, and light does not penetrate easily to the ground. The increase in light penetration offered by diffuse light conditions allows for greater photosynthetic rates for plants on the forest floor, particularly for seedlings (Johnson & Smith 2006).

Sun-flecks are an important source of light for understory plants, especially those species that specialize in gap colonization. Leaves can make short term adjustments to these short bursts of high irradiance, allowing the photosynthetic apparatus to take advantage of this light source. Sun-flecks can provide up to 90% of the light for daily carbon gain in understory plants (Valladares et al. 1997; Leaky et al. 2005). Increases in irradiance that benefit photosynthetic carbon gain are also associated with increased leaf temperature and a greater vapor pressure deficit that could otherwise limit gains (Young & Smith 1979, 1983; Chazdon 1991). Aside from these infrequent, short bursts of direct light, photosynthetically active radiation in the understory is primarily diffuse.

Community-level effects of diffuse light

The eruption of Mt Pinatubo in 1991 brought attention to the relationship between the directionality of light and photosynthesis. During the eruption, light-scattering

aerosols and volcanic ash were spewed into the atmosphere. As these diffusive particles and gases distributed evenly through the atmosphere light at the Earth's surface became more diffuse. This eruption provided a unique opportunity for the scientific community to test the effects of increased proportions of diffuse light on community-level productivity (Gu *et al*. 1999, 2003; Farquhar & Roderick 2003). Various research groups monitored the response of forest communities to the increased diffuse light in different plant communities. These research groups observed increased rates of primary productivity by up to 20% compared to pre- and post-volcanic conditions. It was suggested that the increased diffuse light allows for a more even distribution of light within forest canopies (Melillo *et al*. 1993; Hollinger *et al*. 1994; Geider *et al.* 2001; Roderick *et al*. 2001; Gu *et al*. 2003; Krakauer & Randerson 2003; Misson *et al*. 2005; Urban *et al*. 2007; Alton et al. 2007). With a greater total leaf area illuminated, more of the plant tissue in these forests could contribute to carbon gains.

Crop physiologists have recognized the positive effects of diffuse light on plant growth for some time, but these ideas have only recently been applied to natural communities (Horn 1971; Norman & Miller 1971; Norman & Arkebauer 1991). In maize, collimated light is intercepted by leaves at the top of the plant canopy, thereby shading the lower leaves. Under cloudy skies, where more diffuse light is present, light penetrates deeper into the plant canopy as it enters at low angles and is multiply reflected by leaf surfaces. More recently, it has been shown that diffuse light enhances photosynthesis in tree seedlings in a closed-canopy understory (Johnson & Smith 2006), by making the light environment more uniform and homogenous.

Various climate-change models have predicted that global warming will be

accompanied with increases in diffuse light, due to elevated atmospheric water vapor and associated cloud cover (Pounds & Puschendorf 2004; Feddema et al. 2005; Schiermeier 2006). These climate predictions and the community productivity estimates under diffuse conditions emphasize the necessity of understanding leaf-level responses to direct versus diffuse light.

Measurements of photosynthesis in leaves have been done almost exclusively under conditions of direct light, that is directed perpendicularly to the leaf surface (Long & Bernacchi 2003). This represents a unique experimental condition, given that plants usually experience wide variation in the directional quality of incident light in their natural environment.

PHOTOSYNTHESIS: SCALING FROM THE LEAF TO THE LANDSCAPE

Modeling efforts

Various attempts have been made to scale leaf-level photosynthesis to whole canopies. These methods rely on "big-leaf" models, where the entire canopy is modeled as a huge, single leaf. By simplifying an extremely complex system, "big-leaf" models come close to approximating canopy photosynthesis (Sellers et al. 1992; Amthor 1994; Lloyd 1995). Farquhar (1989) later demonstrated that the same equation used to describe cellular-level photosynthesis could be extrapolated to the whole-leaf level and ultimately to the canopy. This type of model estimates the photosynthetic capacity of leaf tissue with the absorption profile. Here, individual leaves of the canopy and individual chloroplasts of leaves are treated equally. So by measuring the absorption of light

through a canopy, similar models could be used for both leaves and whole canopies (Sellers et al. 1992; DePury & Farquhar 1997). To do this the Rubisco activity per unit ground area is based on the sum of the total leaf area for that same unit of ground. Rubisco content is usually estimated by measuring leaf nitrogen levels. Abiotic factors such as temperature, CO_2 concentration, and irradiance are included in these mathematical models as well. This method, however, tends to overestimate photosynthetic rates because each layer of the canopy does not contribute equally. One particularly large source of error for "big-leaf" models was in calculating the amount of radiation absorbed by the canopy. Leaf angle is different for each individual leaf, and with solar angle changing throughout the day the relationship between leaf angle and light interception is a constantly changing variable. This is one of the main sources of error in this type of model. Following the "big-leaf" models were "multi-layer" models that treated each layer of the canopy differently. DePury & Farquhar (1997) also developed a canopy photosynthesis model based on multi-layer models. This model integrated the illuminated and shaded portions of the canopy as layers, which then acts again as a "big-leaf" model, but is much more accurate and simplified. This model also accounts for the variation in photosynthetic capacity throughout the canopy, and when combined with sun and shade layer integration, yields a very robust canopy model that is still popular.

SUMMARY

The major objective of this dissertation is to explore the effects of the directionality of light on leaf-level absorption and utilization for photosynthesis. Changes in the angular distribution of light at the community-level has been shown to significantly affect productivity, yet no studies exist that address leaf-level processes. Specifically, this body of work aims to establish experimental data in the following areas:

1. To determine if direct and diffuse light are reflected, transmitted, and absorbed differently by leaves, and if epidermal cell structure has a differential effect on the transmission of direct vs. diffuse light.

2. To examine the effects of direct vs. diffuse light on leaf-level photosynthesis, and compare those results with the community level measurements that already exist.

3. To measure light absorption profiles inside leaves irradiated with direct, diffuse, and low-angle light in plants grown in sun and shade conditions. Leaves will be irradiated with red, green, and blue monochromatic light to determine what effect wavelength has on light penetration of varying directional quality.

FIGURES

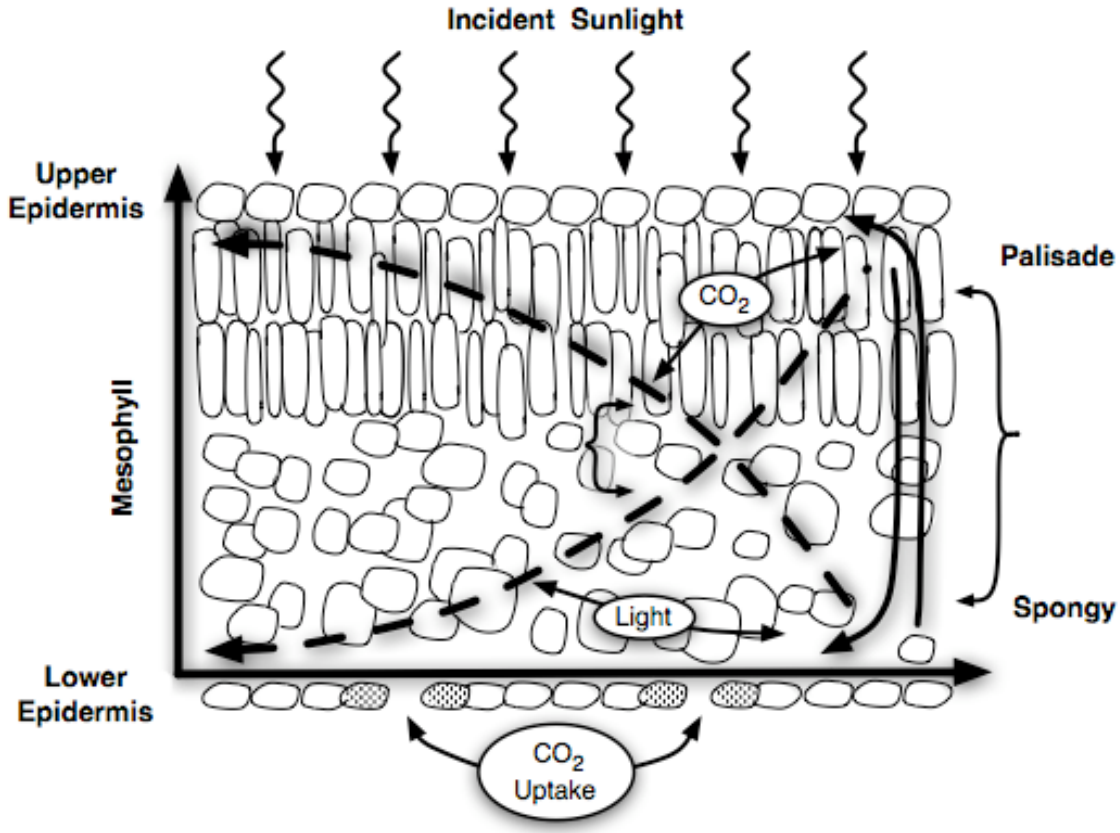

Figure 1. 1 A cross-section of a typical leaf showing opposing gradients of internal light and CO_2 when sunlight is incident on the upper surface and stomata are present predominantly on the lower surface. Two pairs of hypothetical curves are drawn: one pair (dashed lines) shows strong gradients that generate a narrow zone of overlap (indicated by small bracket) between high light and CO_2, and another pair (solid lines) shows smaller gradients that generate a broader zone of overlap (large bracket) between high light and CO_2. A broader zone of overlap would generate greater photosynthesis per unit leaf biomass, which may be a fundamental driving force in evolution of leaf form (From Smith et al. 1997).

CHAPTER 2: MEASUREMENT OF THE REFLECTANCE, TRANSMITTANCE AND ABSORPTANCE OF DIRECT AND DIFFUSE LIGHT

DO EPIDERMAL LENS CELLS FACILITATE THE ABSORPTANCE OF DIFFUSE LIGHT?

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ABSTRACT

Many understory plants rely on diffuse light for photosynthesis because direct light is usually scattered by upper canopy layers before it strikes the forest floor. There is a considerable gap in the literature concerning the interaction of direct and diffuse light with leaves. Some understory plants have well-developed lens-shaped epidermal cells, which have long been thought to increase the absorption of diffuse light. To assess the role of epidermal cell shape in capturing direct vs. diffuse light, we measured leaf reflectance and transmittance with an integrating sphere system using leaves with flat (*Begonia erythrophylla*, *Citrus reticulata*, and *Ficus benjamina*) and lens-shaped epidermal cells (*B. bowerae*, *Colocasia esculenta*, and *Impatiens velvetea*). In all species examined, more light was absorbed when leaves were irradiated with direct as opposed to

diffuse light. When leaves were irradiated with diffuse light, more light was transmitted and more was reflected in both leaf types, resulting in absorptance values 2–3% lower than in leaves irradiated with direct light. These data suggest that lens-shaped epidermal cells do not aid the capture of diffuse light. Palisade and mesophyll cell anatomy and leaf thickness appear to have more influence in the capture and absorption of light than does epidermal cell shape.

Key words: absorptance; diffuse light; epidermal focusing; lens cells; optics; papillose cells; reflectance; transmittance.

INTRODUCTION

The forest floor beneath a dense canopy is a unique environment for understory plants. The light regime is typically diffuse and light intensities are much lower compared to the primarily intense direct light received at the top of the canopy. Direct light can penetrate to the understory through gaps in the canopy, appearing as sun flecks (Smith et al., 1989; Pearcy, 1990). Aside from these infrequent, short, intense bursts of direct light, plants in the understory rely on diffuse light for photosynthesis. Crop physiologists observed many years ago that photosynthesis within canopies increases under diffuse light (Norman and Miller, 1971), and more recently remote sensing research has shown that community level productivity of forests also increases under diffuse light conditions (Roderick et al., 2001; Farquhar et al., 2003; Gu et al., 2003). Climate change scenarios suggest an increase in diffuse light coupled with more moisture in the atmosphere (Geider and DeLucia, 2001; Pounds and Puschendorf, 2004). Therefore, it has become

increasingly important to understand how direct and diffuse light penetrates leaves and how the directional quality of light affects photosynthesis. Leaf epidermal cells constitute an important boundary between the mesophyll and external environments, and they have evolved to serve many purposes such as retaining water, controlling transpiration and CO_2 uptake, repelling water, and discouraging predation by insects (Bone et al., 1985). Epidermal cells of most leaves provide a clear window for light to reach the mesophyll where it is absorbed for photosynthesis. Although usually transparent and free of chloroplasts, the epidermis is often pigmented by anthocyanins, which are synthesized in response to environmental stress (Bone et al., 1985) or as part of normal plant growth and development in special habitats (Lee et al., 1979; Lee and Graham, 1986). Epidermal cells also contain UV-absorbing compounds, which protect mesophyll cells against harmful short wave radiation (Smith et al., 1997; Turunen et al., 1999; Mazza et al., 2000). In addition to pigments, which affect the spectral quality of the transmitted light, epidermal cell shape influences the amount of light that enters a leaf, primarily through lens action. Most epidermal cells have a convex shape that focuses light as it passes into a leaf (Vogelmann, 1993). These cells are fairly widespread throughout the plant kingdom, though they are typically associated with tropical understory herbs and plants that grow in areas with high moisture (Bone et al., 1985). In the most striking examples where epidermal cells are conical or even papillose, leaves have a velvet appearance when viewed from above and a satin sheen when viewed from one side. The focal properties of these cells have been described (Bone et al., 1985; Gorton and Vogelmann, 1996; Vogelmann et al., 1996), but their functional significance remains to be determined. One hypothesis is that focusing light in the mesophyll might create a more favorable light

environment for photosynthesis for at least some of the chloroplasts. But it is difficult to envision how this would be advantageous because adding light to some chloroplasts means that light is taken away from others (Bone et al., 1985). Another idea is that conical epidermal cells make the leaf surface more hydrophobic (Wagner et al., 2003; Bhushan, 2006), thereby reducing the ability of pathogens to colonize the leaf surface and also keeping the stomata clear for gas exchange. These hypotheses are not mutually exclusive, and a third possibility is that these cells might facilitate the capture of the diffuse light prevalent under forest canopies. When light strikes a flat surface, some of it is reflected by specular (mirror-like) reflection. The more oblique the light, the more is reflected, and light that barely glances a flat surface will be almost completely reflected. Adding conical cells increases surface roughness, which could aid the capture of low angle light and increase the amount of usable light in the understory for photosynthesis. The purpose of this study was to test this idea by measuring the differences in reflectance and transmittance of both direct and diffuse light in leaves with two different types of epidermal cell shape—flat and conical. If leaves with conical epidermal cells reflect less diffuse light than flat leaves, then this would support the hypothesis that conical cells aid in the capture of light. To the best of our knowledge, no studies have addressed whether direct and diffuse light is captured similarly by leaves. Here we report experimental results using newly developed instrumentation that makes it possible to measure reflectance from leaves irradiated with diffuse light.

MATERIALS AND METHODS

Plant species

Plants chosen for study with convexly shaped leaf epidermal cells were *Begonia bowerae* Ziesenh., *Colocasia esculenta* (L.) Schott., and *Impatiens velvetea*. Species with topographically flat epidermal cells were *Begonia erythrophylla* Neum., *Citrus reticulata* Blanco, and *Ficus benjamina* L. Plants were grown in a glasshouse under 500-1400 µmol m^{-2} s^{-1} and a 21˚/18.5˚ C day/night temperature. Mature leaf samples were collected and stored in a moist, sealed plastic bag until measurements were conducted (less than 30 min). Leaf disks were taken for optical measurements (described later) and adjacent leaf tissue was sampled for anatomical measurements of epidermal, palisade, and mesophyll cell dimensions as well as total leaf thickness and a measure of the curvature of epidermal cells. Twenty measurements were made for each species for each anatomical attribute (Table 2.1, Fig. 2.1). Cell dimensions and tissue layer thickness were measured using an ocular micrometer calibrated against a stage micrometer. Epidermal cell surface angle was assessed by measuring the angle bisecting the apex of the cell using the ‘‘measure’’ tool in Adobe Photoshop CS (Adobe Systems Incorporated, San Jose, California, USA) (Fig. 2.2). Twenty epidermal cells were assessed for this measurement per species. Optics measurements under direct and diffuse light—Reflectance and transmittance spectra were measured from leaf samples using an integrating sphere (Spectralon interior, 15.25 cm diameter, Labsphere, North Sutton, New Hampshire, USA). White light from the xenon arc lamp (150 W, Photon Technology International, Monmouth Junction, New Jersey, USA) passed through an entrance port and was directed

to an exit port on the opposite side of the sphere. For measuring transmittance, a leaf disk, 2.38 cm in diameter was cut with a cork borer and affixed to the entrance port. For measuring reflectance, the leaf sample was attached to the exit port. Measurements were calibrated against a 99% reflectance standard (Spectralon SRS-99–010, Labsphere). Light in the sphere was transmitted through a fiber optic cable, which was attached to a port 90 degrees from the entry port, then directed to a spectrometer (S2000, Ocean Optics, Dunedin, Florida, USA). Reflectance (R), transmittance (T), and absorptance (A) were calculated as described previously (Gorton et al., 2001) according to the relationship:

$$R + T + A = 1;$$

where 1 is the total fractional quantity of light that strikes a leaf.

Optical properties of leaves irradiated with diffuse light

Measurements of leaf reflectance under diffuse light required special instrumentation that will be described in detail elsewhere. Briefly, a dual-beam integrating sphere spectrometer was constructed in which monochromatic light was split into two beams, each of which was directed into an integrating sphere, one for sample and one for reference (Model CA-06050–000, Labsphere). The light was chopped such that it was alternately directed into the sample and reference spheres, and light was detected in each sphere by a bifurcated optical cable attached to a photomultiplier. The signal from the photomultiplier was sent to a lock-in amplifier. Lock-in detection allowed measurement of small leaf reflectance signals against the large amount of background light within the

sphere. Reflectance was measured by placing a leaf disk on a port, located 90˚ from the entrance port, such that the disk was irradiated with diffuse light emanating from the interior of the sphere. With the leaf sample in place, measurements were made at each wavelength as the monochromator advanced from 400–700 nm. Similar measurements were made with the port left open (baseline, B_l) and in the presence of a 99% reflectance standard (S_l). Total reflectance (R_tl) was calculated as:

$$R_{tl} = [(R_{s1}-B_1)/(S_1-B_1)] \times K_1,$$

where K_l = spectral calibration constant at each wavelength for the reflectance standard.

For measuring the amount of light that was transmitted through leaves when they were irradiated with diffuse light, diffuse incident light was created by directing white light into an integrating sphere as described earlier. A leaf sample was placed on an exit port of the sphere where it was irradiated with diffuse light, and then a second detector integrating sphere moved in place such that it captured the light that was transmitted through the leaf (T_s). Light was measured in the detector sphere through a fiber optic cable and spectrometer as described earlier. A reference baseline was measured with no sample in place (T_r). Transmittance (T_d) was calculated as

$$T_d = T_s / T_r,$$

Representative values of the reflectance, transmittance, and absorptance of direct and diffuse light have been provided.

RESULTS

Leaf anatomy of study species

Clear differences in epidermal cell shape were evident between the two study groups. Leaves with perfectly flat epidermal cells would theoretically have a curvature value of 180˚, parallel with the leaf surface, while leaves with curvature values of 130˚ would have more lens-like cells. Species with lens cells had lower curvature angles, with an average of 30.5˚ less curvature than leaves with a flat surface. Leaf anatomical characteristics varied within the study group, and no clear trends were evident between the two study groups regarding palisade, mesophyll, or total leaf thickness (Table 2.1, Fig. 2.1).

The two *Begonia* species, which had similar leaf morphology, offered the opportunity to measure the effect of the epidermis on leaf reflectance, the primary difference being the presence or absence of lens-shaped epidermal cells. For these two species, the general trends observed in this study apply. *Begonia erythrophylla* responds like the other glossy plants, while *B. bowerae* responds like the other plants with lens cells.

Reflectance of direct and diffuse light

Our measured values for direct reflectance fall within the range for most leaves under direct light, with an average reflectance of 3.5% and 4.0% at wavelengths of maximum absorption in the blue (450 nm) and red (650 nm) regions, respectively, and 7.8% and

45.8% in the far (700 nm) and infrared (750 nm), respectively, where there is minimal absorption. Reflectance within the green was variable, depending upon the amount of pigmentation and leaf anatomy, and ranged from 4.2% to 17.4% at 550 nm in our leaves. Reflectance at all wavelengths studied in both diffuse and direct light was typically lower in plants with epidermal lens cells than those without epidermal lens cells (Fig. 2.3) (*B. erythrophylla*: 5.8% and 6.0% diffuse reflectance, and 4.7% and 4.5% direct reflectance at 450 nm and 500 nm, respectively; *B. bowerae*: 3.2% and 4.4% diffuse reflectance, and 2.1% and 3.1% direct reflectance at 450 nm and 500 nm, respectively). In leaves of both surface types, diffuse light was typically reflected more than direct light between 400 nm and 650 nm. Beyond 650 nm, direct light was reflected less than diffuse light in all leaves except the two *Begonia* species. Diffuse light was consistently reflected more than direct light across the entire spectrum.

Transmittance of direct and diffuse light

Diffuse light was transmitance through all leaves slightly less than direct light (Fig. 2.4). *Impatiens velvetea* had the lowest transmittance values across the spectrum for diffuse light (2.0, 1.9, and 3.5% at 450, 500, and 700 nm, respectively), while *C. reticulata* had the lowest values for direct light at 450 and 500 nm (0.1% and 0.1%, respectively). The highest transmittance values for direct light at 450 nm and 500 nm were in *F. benjamina* (4.9% and 4.9%, respectively), and the highest transmittance values for diffuse light at 450 nm, 500 nm, and 700 nm were in *B. bowerae* (2.5%, 5.3%, and 17.6%, respectively). When the two *Begonia* species were compared, *B. erythrophylla*

had higher transmittance of diffuse light at 450 nm (5.8% vs. 3.2% for *B. bowerae*) and 500 nm (6.0% vs. 4.4% for *B. bowerae*) as well as of direct light at 450 nm (4.7% vs. 2.1% for *B. bowerae*) and 500 nm (4.5% vs. 3.1% *for B. bowerae*). Throughout this range, both types of light were typically transmitted the most around 500 nm and above 700 nm.

Absorptance of direct and diffuse light

Absorptance of direct light was typically slightly higher or equal to diffuse light absorptance throughout the entire spectrum for plants with and without lens cells, while plants with lens cells absorbed slightly less diffuse light (Fig. 2.5). Absorption was highest at 450 nm under direct and diffuse light for all species. Absorptance was typically lowest in the 525–550 nm range of the visible spectrum for all species. The only remarkable differences (greater than 1.5%) in diffuse or direct light absorptance occurred in *C. esculenta*, *I. velvetea*, and *C. reticulata* at 550 nm (16.5%, 12.5%, and 5.8%, respectively). Most species absorbed more direct light than diffuse light across the photosynthetically active wavelengths (400–700 nm), except for *F. benjamina* around 550 nm and *B. erythrophylla* at 625 nm.

DISCUSSION

The optical properties of the plants in this study were consistent with the ranges of transmittance, reflectance, and absorptance values observed for other species (Woolley, 1971; Gausman and Allen, 1973; Knapp and Carter, 1998). The typical transmission

spectrum of leaves has a minimum in the blue wavelengths, a transmission peak within the green wavelengths, and maximum transmission in the far-red and infrared wavelengths.

These data suggest that because no consistent differences in the reflectance, transmittance, or absorptance of direct and diffuse light were observed between leaves with and without lens cells, lens cells do not appear to aid in the absorptance of diffuse light as was originally hypothesized. The presence of lens cells appeared to negatively influence the absorptance of diffuse light in two of the species (*C. esculenta* and *I. velvetea*). The velvety sheen that we refer to is probably a visual confirmation that multiple reflections occur in the epidermal cell layer of leaves with well-developed lens cells (Fig. 2.6), and those multiple reflections could be responsible for the decreased absorptance of diffuse light in the green wavelengths.

Overall, *I. velvetea* had the lowest transmittance and reflectance values for both diffuse and direct light across the entire spectrum. This is most likely due to the dark green pigmentation, which can appear almost black depending on growing conditions. The leaf pigmentation of this species is fairly plastic, with smaller and lighter-colored leaves in full sun, and broad, dark-colored leaves in heavy shade.

The causes of the greatest differences (greater than 1.5%) in diffuse or direct light absorptance occurring at 550 nm in *C. esculenta*, *I. velvetea*, and *C. reticulata* are not clear, although the leaves of *C. esculenta* and *C. reticulata* were very thin. The thicker leaves of the other plants may be able to absorb more direct light at wavelengths near 550 nm. It appears as though this phenomenon was a function of leaf thickness and possibly epidermal cell structure because the trend occurred in both study groups, those with and

without lens cells.

The most striking result from this study was the unequal reflectance of diffuse and direct light independent of epidermal cell structure. With a greater proportion of diffuse light reflected from the surface of all leaves in this study, less light enters the leaf for photosynthesis. The unequal absorptance of direct and diffuse light and the extent to which a change in the directional quality of light affects photosynthesis at the leaf level is not yet known, but community level productivity in diffuse light has been estimated to be higher than in direct light, presumably because light is distributed more evenly within the canopy (Roderick et al., 2001; Farquhar and Roderick, 2003; Gu et al., 2003).

Pigment distribution, leaf morphology, and cellular arrangement appear to have significantly more effect than epidermal cell shape on the reflectance, transmittance, and absorption of diffuse light. Lens cells may then be more important for the focusing of direct light (Vogelmann et al., 1996) or for other reasons such as storing water and improving the hydrophobicity of the leaf surface. The development of these lens-shaped cells in understory tropical species may be primarily related to chance opportunities to exposure to direct light when sun flecks penetrate to the ground level of the forest. In addition, plants with these types of cells typically have an extremely hydrophobic surface, and convexly shaped cells increase water repellency (Wagner et al., 2003; Bhushan and Jung, 2006).

Lens cells are often found on both the abaxial and adaxial sides of the leaf, and freeing either surface of a film of water may be critical for reducing the presence of fungal and bacterial pathogens, as well as for promoting gas exchange. Knowing how diffuse light affects photosynthesis will ultimately help determine the importance of the

percentage of diffuse or direct light a plant receives. Technical limitations have kept such measurements from being performed, but this is a promising area for future research.

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TABLES

Table 2. 1 Physical properties and dimensions of epidermal cells by species

Table 1. Physical properties and dimensions of epidermal cells by species.

Species	Curvature	Adaxial Epidermal Cell Thickness (µm)	Palisade Thickness (µm)	Spongy Mesophyll Thickness (µm)	Leaf Thickness (µm)
Begonia erythrophylla	172 °	55	51	78	760
Citrus reticulata	177 °	9	74	167	262
Ficus benjamina	178 °	15	76	82	218
Begonia bowerae	149 °	63	55	76	455
Colocasia esculenta	148 °	21	88	63	252
Impatiens velvetea	146 °	45	197	248	391

FIGURES

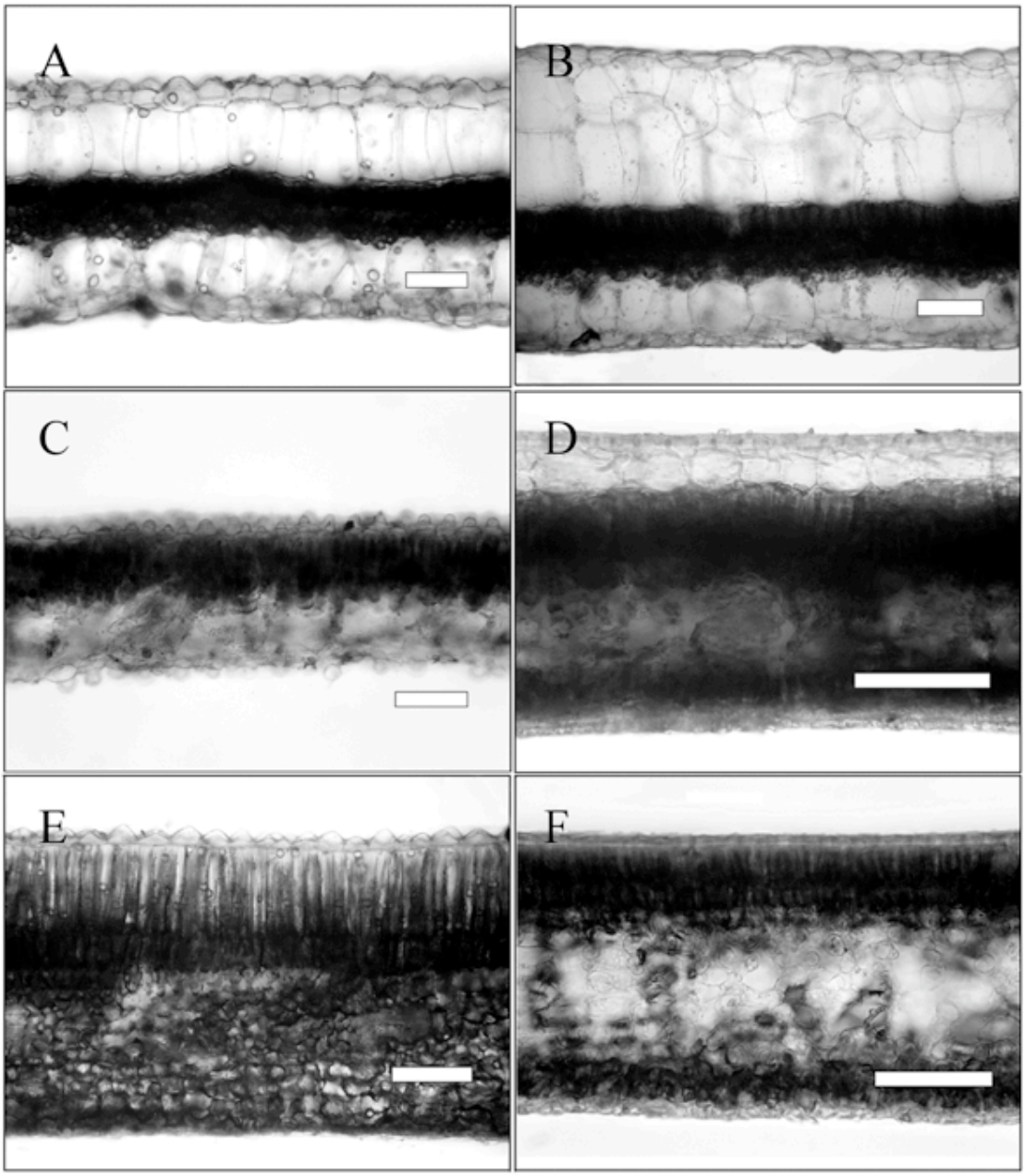

Figure 2.1 Cross sections of leaves showing varying epidermal cell characteristics and leaf anatomy. A) *Begonia bowerae* B) *B. erythrophylla* C) *Colocasia esculenta* D) *Ficus benjamina* E) *Impatiens velvetea* F) *Citrus reticulata*. Scale Bar = 100 µm.

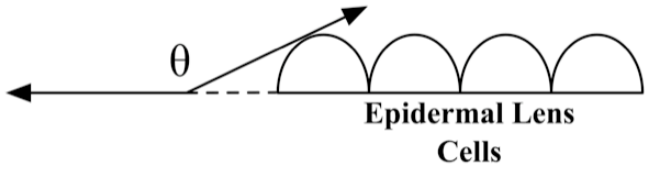

Figure 2.2 Method for quantifying epidermal cell curvature (Θ), where Θ was determined by analyzing a digital image of a cross section of each species using the "measure" tool of Adobe Photoshop CS to calculate the angle of cell curvature from parallel to the leaf surface.

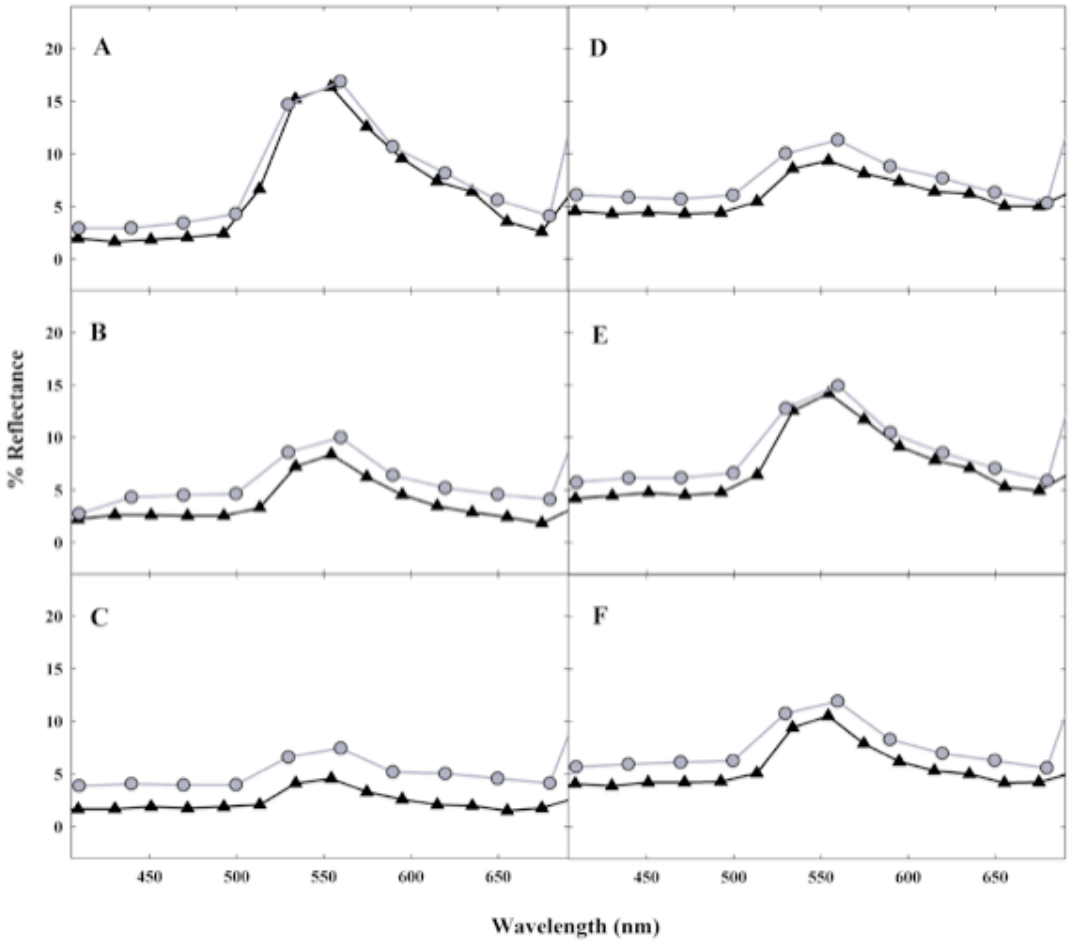

Figure 2.3 Percentage of reflected light from leaves with either (A-C) flat or (D-F) lenticular epidermal cells after irradiation with direct (black lines, black triangles) or diffuse light (black lines, open circles). (A) *Begonia bowerae* (B) *Colocasia esculenta* (C) *Impatiens velvetea* (D) *B. erythrophylla* (E) *Ficus benjamina* (*F) Citrus reticulata*. Graphs show representative data.

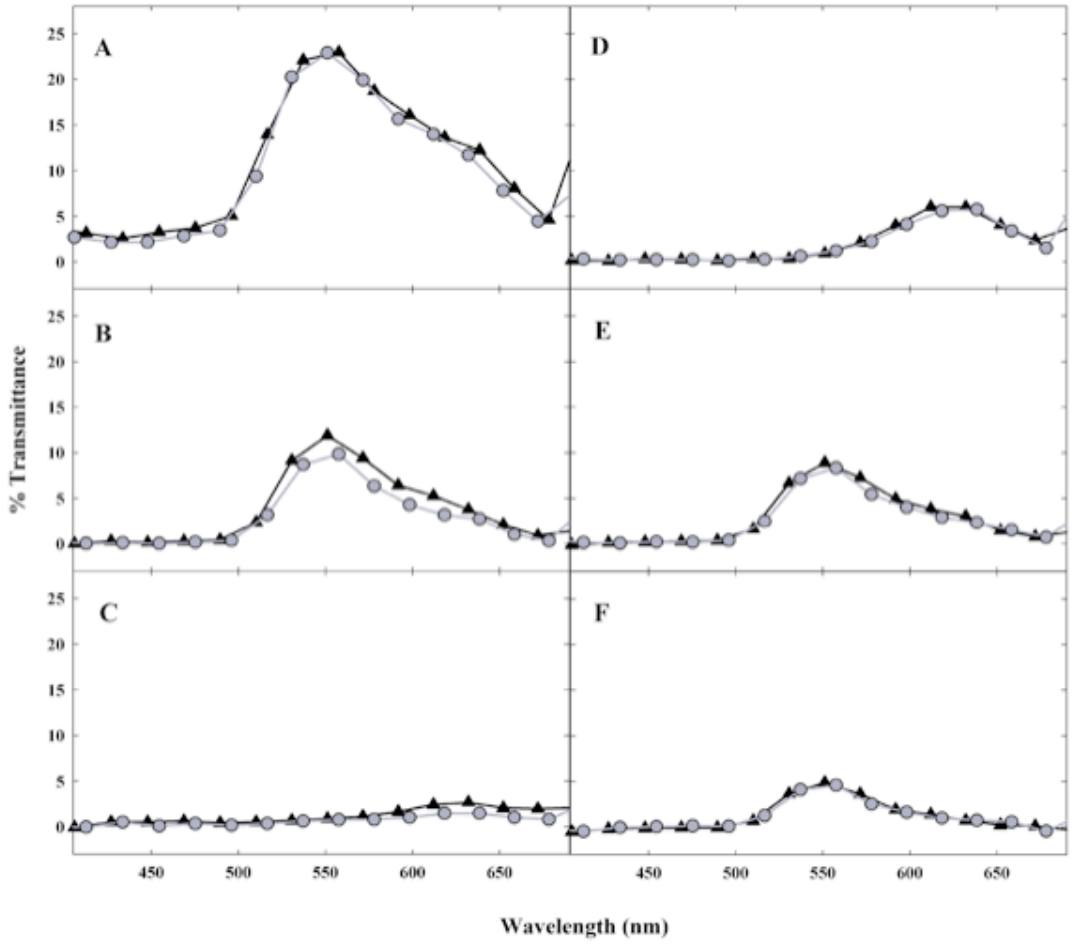

Figure 2.4 Percentage of transmitted light from leaves with either (A-C) flat or (D-F) lenticular epidermal cells after irradiation with direct (black lines, black triangles) or diffuse light (black lines, open circles). (A) Begonia bowerae (B) *Colocasia esculenta* (D) *B. erythrophylla* (E) *Ficus benjamina* (F) *Citrus reticulata*. Graphs show representative data.

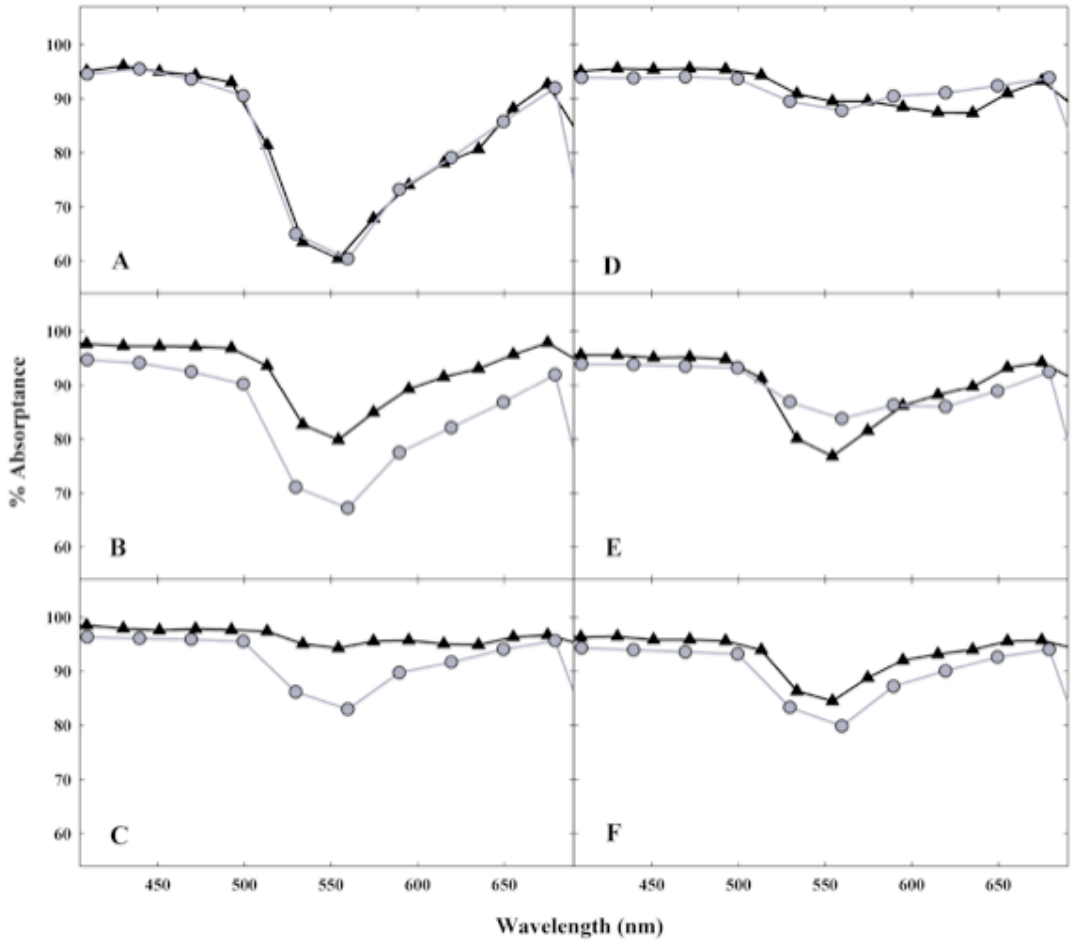

Figure 2.5 Percentage of absorbed light from leaves with either (A-C) flat or (D-F) lenticular epidermal cells after irradiation with direct (black lines, black triangles) or diffuse light (black lines, open circles). (A) *Begonia bowerae* (B) *Colocasia esculenta* (C) *Impatiens velvetea* (D) *B. erythrophylla* (E) *Ficus benjamina* (F) *Citrus reticulata*. Graphs show representative data.

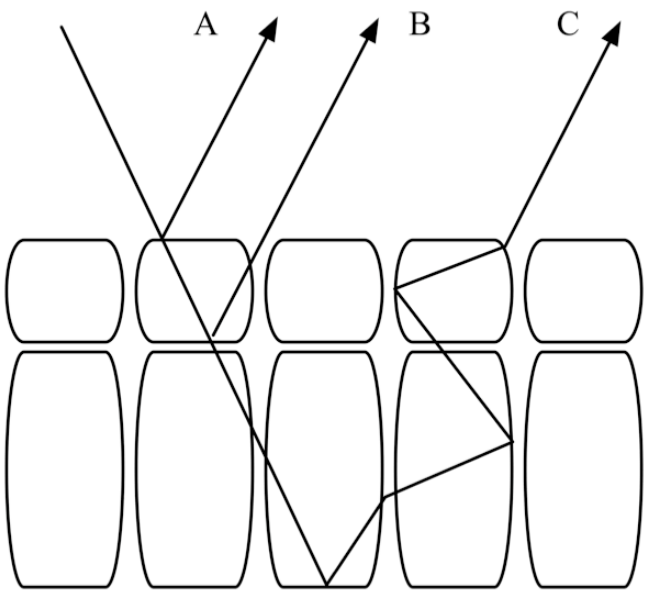

Figure 2.6 Origins of reflected light from a leaf. (A) Mirror-like or specular reflection comes from the leaf surface, whereas (B and C) diffuse reflectance originates from light scattering within the leaf.

CHAPTER 3: EFFECTS OF DIRECT AND DIFFUSE LIGHT ON LEAF-LEVEL PHOTOSYNTHESIS

A NEW PARADIGM IN LEAF-LEVEL PHOTOSYNTHESIS: DIRECT AND DIFFUSE LIGHT ARE NOT EQUAL

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ABSTRACT

Global-change scenarios suggest a trend of increasing diffuse light due to expected increases in cloud cover. Canopy-level measurements of plant-community photosynthesis under diffuse light show increased productivity attributed to more uniform distribution of light within the forest canopy, yet the effect of the directional quality of light at the leaf level is unknown. Here we show that leaf-level photosynthesis in sun leaves of both C_3 and C_4 plants can be 10–15% higher under direct light compared to equivalent absorbed irradiances of diffuse light. High-light grown leaves showed significant photosynthetic enhancement in direct light, while shade-adapted leaves showed no preference for direct or diffuse light at any irradiance. Highlight- grown leaves with multiple palisade layers may be adapted to better utilize direct than diffuse light, while shade leaf structure does

not appear to discriminate light based on its directionality. Based upon our measurements, it appears that leaf-level and canopy-level photosynthetic processes react differently to the directionality of light, and previously observed increases in canopy-level photosynthesis occur even though leaf-level photosynthesis decreases under diffuse light.

Key-word: radiation.

INTRODUCTION

With the eruption of Mt Pinatubo in 1991 and the subsequent increase in light-scattering aerosols in the atmosphere, the scientific community began to fully recognize the potential effects that changes in the directionality of light might have on plant communities on a global scale (Gu *et al*. 1999, 2003b; Farquhar & Roderick 2003; Min 2005). The emission of volcanic aerosols into the atmosphere produced a layer of diffusive particles that interacted with direct-beam radiation from the sun to increase the proportion of diffuse light irradiating the earth's surface. Various research groups monitored the response of forest communities to the increased proportion of diffuse light, and observed increased rates of photosynthesis, possibly caused by more even distribution of light within the leaf canopy (Melillo *et al*. 1993; Hollinger *et al*. 1994; Geider *et al.* 2001; Roderick *et al*. 2001; Gu *et al*. 2003a; Krakauer & Randerson 2003; Misson *et al*. 2005; Urban *et al*. 2007). More recently, it has been shown that diffuse light enhances photosynthesis in tree seedlings in a closed-canopy understory (Johnson & Smith 2006). Crop physiologists have recognized the positive effects of diffuse light on

plant growth for some time, but these ideas have only recently been applied to natural communities (Horn 1971; Norman & Miller 1971; Norman & Arkebauer 1991). Various climate-change models have predicted future increases in diffuse light due to elevated atmospheric water vapour due to increased cloud cover (Pounds & Puschendorf 2004; Feddema *et al.* 2005; Schiermeier 2006). These climate predictions and the community productivity estimates under diffuse conditions emphasize the necessity of understanding leaf-level responses to direct versus diffuse light. Whether these effects scale from the community level to the leaf level is still unknown, but a recent study shows that some leaves absorb approximately 2–3% less diffuse light than collimated light, suggesting that direct and diffuse light affect photosynthetic processes differently from the leaf to landscape (Brodersen & Vogelmann 2007).

Measurements of photosynthetic carbon exchange in leaves have been done almost exclusively under conditions of direct light (Long & Bernacchi 2003). This represents a unique experimental condition, given that plants usually experience wide variation in the directional quality of incident light in their natural environment. On a clear day, sunlight arrives in beams at the earth's surface, with approximately 85% of the light being direct and 15% of the light scattered by the atmosphere (Bird & Riordan 1986). On a cloudy day, nearly 100% of the incoming light is diffuse, as clouds, haze or fog scatters the light before it reaches the earth's surface. Under direct beam light, leaves at the top of a canopy will usually be saturated photosynthetically while leaves located in the lower canopy are shaded and light limited. Diffuse light distributes photosynthetically active radiation more uniformly to all leaves within a canopy, enhancing the overall rate of photosynthesis (Gu *et al*. 2003a). However, to the best of our knowledge no information

is available about the relative ability of individual leaves to utilize direct versus diffuse light for photosynthesis, leaving open the question of how best to scale photosynthetic rates of individual leaves to the canopy level under varying light conditions. Previous research describing anatomical features adapted for direct light such as palisade tissue acting as light conduits and the focusing of light by epidermal lens cells, suggest that different leaf-level photosynthetic responses to direct and diffuse light may exist (Vogelmann *et al*. 1996; Smith *et al*. 1997). Here we present results that suggest that to leaves, direct and diffuse light are not equal: leaves that develop under direct highlight conditions can use direct light more effectively than diffuse light, while those that develop under diffuse, lowlight conditions use diffuse and direct light equally well.

MATERIALS AND METHODS

Greenhouse growth conditions

Helianthus annuus (C_3) and *Amaranthus retroflexus* (C_4) were grown from seed in a greenhouse. One group of plants was grown with supplemental lighting (400 W HPS lamps) and a second group was grown without supplemental lighting. Light levels for each treatment were characterized by measuring PPFD at the plant crown level between 0900 and 1900 h using four LI-190 PPFD sensors and a Campbell
CR-23x datalogger (NB Li-Cor Inc., Lincoln, NE, USA; Campbell Scientific Inc., Logan, UT, USA). We averaged irradiance values from 3August to 13August 2006 between 0900 and 1900 h as representative growth conditions. Average daytime temperature in

the greenhouse was maintained at 30 °C. All plants were randomly repositioned daily in both light treatments to minimize the effects of any nonuniformity of light on the greenhouse benches.

The degree of collimation of light for both treatments was determined by measuring the ratio of direct to diffuse light using a BF3 Sunshine Sensor that measured the direct beam and diffuse components of incoming light at the leaf surface (Delta-T Devices Ltd., Cambridge, UK). In the greenhouse, these measurements were made in the morning (1030 h) and in the afternoon (1600 h).

Gas-exchange measurements

Plants 4–6 weeks old with fully expanded leaves on the sixth to eighth node were chosen for gas-exchange measurements. Six different light-response curves were measured for plants in each group, using a different plant for each measurement, under both direct and diffuse light using an LI-6400 portable photosynthesis system (LI-COR Inc.), and plants were allowed to acclimate at 500 μmol m^{-2} s^{-1} PPFD before instantaneous photosynthesis rates were measured at 0, 50, 100, 200, 300, 500, 750, 1000 and 1500 μmol m^{-2} s^{-1} PPFD for either direct light or diffuse light. Light-response curves were also performed by beginning at the highest irradiance and decreasing to 0 μmol m^{-2} s^{-1} PPFD as described by Björkman (1981), yet each method yielded similar results. These irradiances were then corrected for the amount of light actually absorbed by the leaf, as measured using an integrating sphere system to determine the reflectance and transmittance of direct and diffuse light as described in Brodersen & Vogelmann (2007).

Direct and diffuse lighting for gas-exchange measurements

Direct light was generated from a quartz halogen lamp (82 V, 300 WEXR) in a projector (Kodak Ektagraphic III E Plus; Eastman Kodak Company, Rochester, NY, USA) pointed perpendicular to the leaf surface (Fig. 1a). Specific irradiances were achieved using metallic neutral-density filters (Melles Griot, Inc., Rochester, NY, USA). Diffuse light was produced by directing light from the projector into a port on the equator of a 20.32-cm-diameter custom-made integrating sphere coated with a 1-mm-thick coating of barium sulphate white reflectance paint (Fig. 1b) (Munsell Color, New Windsor, NY, USA). The LI-6400 sensor head was equipped with a fused silica dome window (OL ISA-2DW Dome Window, Optronic Laboratories, Inc., Orlando, FL, USA) to ensure uniformity of passage of light into the cuvette to the leaf surface. The fused-silica dome was placed in a port located at the south pole of the sphere where the light was diffused by multiple reflections within the sphere; with this configuration the leaf surface was 17 mm below the port, a 2 mm difference from the Li-Cor 2 × 3 cm standard leaf chamber. Spectral irradiance was measured with a spectrometer (HR4000, Ocean Optics, Inc., Dunedin, FL, USA) calibrated against a NIST-traceable standard lamp (Mikropack DH2000; Ocean Optics, Inc.) to confirm uniformity between the light treatments. We used two methods to measure the degree of collimation of the light sources in the lab. Firstly, we used the BF3 sensor that we used in the greenhouse measurements to give us a direct comparison of the degree of light collimation that the plants received during growth with that they received during photosynthesis

measurements with our direct source. However, the BF3 sensor was too large to be completely illuminated by light coming out of the integrating sphere, and we also wanted to measure the degree of collimation directly inside the gas-exchange cuvette when illuminated by both direct and diffuse sources. For these measurements we used a single optical fibre (FHP 100/140/170 fused silica, numerical aperture 0.22 (12.7° acceptance angle, Polymicro Technologies, Inc., Phoenix, AZ, USA) glued into the eye of a needle that was mounted on-axis at the end of a metal rod attached to the centre of a calibrated rotation stage. We then removed the bottom of the gas-exchange chamber and positioned the fibre at leaf level. Light from the optical fibre was routed to a photomultiplier assembly (R3788 tube, model C1053-01 socket assembly; Hamamatsu, Shimokanzo, Japan), powered by a high-voltage power supply (Model 215, Bertan Associates, Inc., Valhalla, NY, USA), and the output was sent to a strip-chart recorder. We recorded the signal every 5 degrees as we rotated the fibre through 180 degrees, from horizontal, through vertical, and back to horizontal. We repeated the measurements with the fibre oriented perpendicular to its original orientation, again rotating it from horizontal, through vertical, and back to horizontal. Values presented are an average of data obtained as the fibre rotated through two perpendicular planes corresponding to the *x*- and *y*-axes of the chamber window.

We used the same optical fibre, oriented vertically, to assess the uniformity of light within the chamber. We took nine measurements along a 3 × 3 grid within the chamber, one central measurement and eight around the window about 2 mm from the edge.

Microscopy

Leaf anatomical measurements were made from images of three cross sections taken from three leaves from each plant using image-analysis software (Image Pro Plus, Media Cybernetics, Inc., Silver Spring, MD, USA). Statistical analysis was performed using Sigma Plot (Systat Software, Inc., Point Richmond, CA, USA).

RESULTS

Characterization of light sources

During plant growth, mean maximum irradiance levels in the greenhouse were 450 and 130 μmol m^{-2} s^{-1} PPFD for the high-light and low-light treatments, respectively. The direct:diffuse ratios as measured with the BF3 sensor for the highlight and low-light treatments were 0.31 and 0.21 (at 1030 h) and 1.03 and 0.40 (at 1600 h).

The direct:diffuse ratios measured with the BF3 sensor for the direct light source used for laboratory gas-exchange measurements was 60; the BF3 sensor was too large to allow a corresponding measurement of the diffuse source.

Measurements made with an optical fibre indicated that angle of the cone of light striking the leaf surface from the direct light source was 22° at half maximum intensity; the corresponding angle for the diffuse light from the integrating sphere was 105°.

During gas-exchange experiments, we used the light sensor in the chamber, which was calibrated against a centrally positioned PAR sensor. Field uniformity measurements allowed us to evaluate how accurate those measurements were. The diffuse light was

more uniform than the direct light. If the signal in the centre of the cuvette window was 100%, the average signal for the grid of nine points within the window was 98 ± 2% for the diffuse source and 90 ± 13% for the collimated source. Light measurements during gas exchange were based on calibrations against a centrally positioned sensor. Thus, the field uniformity data suggest that the light measurements for the direct source are overestimates of the irradiance striking the entire leaf surface within the cuvette. Spectral quality analysis of the direct and diffuse light sources yielded nearly identical data from 400 to 650 nm (Fig. 3.2). The diffuse light source showed a slight enrichment in wavelengths beyond 650 nm.

Photosynthesis measurements

Representative examples of C_3 (*H. annuus*) and C_4 (*A. retroflexus)* broad-leafed plants grown in high-light conditions showed elevated rates of photosynthesis under direct light compared to diffuse light (15.6 and 9.5% higher for *H. annuus* and *A. retroflexus*, respectively). This was especially evident when plants were neither light-limited nor light inhibited (500–1000 μmol m^{-2} s^{-1} PPFD), where there was a clear preference for direct light (Fig. 3.3a,c). Plants grown in low light showed no significant difference photosynthetically when illuminated with direct or diffuse light regardless of irradiance: the differences observed in high-light plants disappeared (Fig. 3.3b,d).

Microscopy

We looked for anatomical features of the leaves that might be correlated with the differences in photosynthetic performance we observed. Palisade layers were significantly thicker in both species when grown with supplemental lighting, and high-light-grown *H. annuus* had a double palisade layer, which is typical of leaves grown in high light (Terashima *et al*. 2006) (Table 3.1). Spongy mesophyll thickness was significantly greater in high-light *A. retroflexus*, while *H. annuus* showed no significant difference in mesophyll thickness for plants grown in high and low light.

DISCUSSION

A major finding of this study is that direct and diffuse light can elicit different photosynthetic responses in some leaf forms but not others, indicating that, in addition to irradiance, the directional quality of light can play a role in determining photosynthesis at the leaf level. Experiments with plants grown under high versus low light implicate leaf anatomy in this differential response to light. Leaves grown under supplemental high light developed sun-leaf characteristics, with thicker palisade than leaves grown under low light. Measurements of the directional quality of the light in the greenhouse showed that it was predominantly diffuse and that there were no significant differences in the directional quality of the light between the high- and low-light growth conditions. Thus, the higher irradiance during growth, rather than a difference in the directional quality of the growth irradiance, appears to have led to the formation of leaves that were

predisposed to use direct light more efficiently than diffuse light. The current findings are consistent with the suggestion that palisade cells propagate light deeper into the leaf (Vogelmann 1993; Smith *et al*. 1997). It is unknown whether growing plants under direct versus diffuse light, or under natural light where there are greater extremes in the directional quality of light than found in the greenhouse, would elicit additional photosynthetic effects. Given that direct light from the sun is significantly more collimated than the light source in the lab (<0.5° versus 22°), the photosynthetic effects elicited by direct versus diffuse light in the natural environment could be greater than what we measured in the lab.

Current photosynthesis models consider how excess light is 'wasted' when a leaf's internal light environment does not match its profile of photosynthetic capacity (Buckley & Farquhar 2004; Evans & Vogelmann 2006). Because sun leaves are likely to have greater differences in photosynthetic capacity between upper and lower layers of cells than shade leaves, they are perhaps more likely to exhibit a mismatch between the internal light absorption profile and the profile of photosynthetic capacity when the external light environment changes. Palisade tissue directs collimated light into the interior of the leaf more effectively than diffuse light (Vogelmann & Martin 1993), suggesting that thicker sun leaves may be more predisposed to shifts in their internal absorption profiles, in response to a change in the directional quality of the ambient light, than thinner less anatomically differentiated shade leaves.

The extent to which the differential response to direct and diffuse light occurs among plants is unknown but the finding that it occurs in leaves with both the C_3 (*H. annuus*) and C_4 (*A. retroflexus*) photosynthetic pathways indicates that it is not limited to a species

with a particular photosynthetic pathway or leaf anatomy. Maximum photosynthetic performance in leaves of C_3 plants depends upon a close match between internal light absorption and photosynthetic capacity and the same is true for C_4 plants, except that there are additional constraints imposed by the need to balance metabolic fluxes between mesophyll and bundle sheath cells. Altering the balance between the amount of light absorbed by mesophyll and bundle-sheath cells in *Flaveria bidentis* leaves, by irradiating them with monochromatic blue or green light, showed that carbon fixation was significantly affected by the distribution of light absorption between mesophyll and bundle-sheath cells (Evans 2007). Green light was absorbed most uniformly throughout the leaf whereas blue light was strongly absorbed near the surface, with little light penetrating through the bundle sheath. Consequently, the rate of CO_2 assimilation under blue light was only half that under green light at the same irradiance. Shifts in light absorption within photosynthetic tissues caused by changes in the directional quality of white light would be expected to produce similar results.

Photoinhibition may also contribute to the differences in photosynthesis under direct and diffuse light. For example, under intense direct light conditions, chloroplasts move to periclinal walls, presumably reducing photoinhibition by shading other chloroplasts (Gorton et al. 1999). Under diffuse light, with light arriving from every direction, chloroplast movement to the periclinal walls is not complete (Gorton et al. 2003) so self-shading and photoprotection may not be as effective. Chlorophyll fluorescence measurements under direct and diffuse light could help resolve these issues.

There was a small enrichment of far-red light in our diffuse light source (Fig. 3.2) but otherwise the spectral quality was very similar to the direct light used in experiments. We

tested whether this slight difference might influence our measurements by adding long wavelength light to the direct light source. This supplemental light had no effect on photosynthesis (data not shown). Moreover, if this spectral difference were to have any effect, we would expect it to enhance photosynthesis under diffuse light, a response that is opposite to what was measured. The spectral quality of light varies considerably in nature and is affected by solar angle, clouds, atmospheric aerosols and leaf canopies. Light that is diffused by clouds will have a different spectral composition than light scattered by leaf canopies, which remove most of the visible light, but transmit some green and much of the far red (Dye 2005; Min 2005). Thus, photosynthetic effects in nature caused by change in directional quality are likely complicated by potentially large changes in spectral composition, especially within the photosynthetically active region of the spectrum.

The angle at which light reaches a canopy has long been recognized as an important characteristic of light interception; low-angle direct-beam light striking a leaf canopy is more effective in filling the radiation space within that canopy than light from above (Cowan 1968). The spectral quality of direct light is dependent on solar elevation, where diffuse light is generally independent of solar elevation (Navrátil *et al*. 2007). At the leaf level, it is likely that collimated light that strikes a leaf obliquely at low angles may be less effective for photosynthesis than light that strikes the leaf perpendicularly, especially with changing spectral quality at low solar elevations where the distance light must travel through the atmosphere is greater. This follows from our results where diffuse light, where the vast majority of rays strike a leaf at non-perpendicular angles, was less effective than direct perpendicular light. Leaf development and internal anatomy are

strongly dependent on leaf-level microclimate and the orientation of leaves within a canopy, with the production of horizontally or vertically displayed leaves ultimately determining canopy light interception by changing chlorophyll distribution within leaves (Evans & Vogelmann 2003, 2006). Hence, effects of low-angle light on the photosynthetic responses of canopies may be different than those at the leaf level.

While previous research has shown increases in productivity at the community level under diffuse light, we have shown that leaf-level photosynthetic rates can go in the opposite direction. Our data do not negate community level measurements, but instead show that different processes are acting at different levels within the plant community. Careful observation of plants in their natural environment under both natural and simulated direct and diffuse light is the next logical step in understanding how photosynthesis scales from leaf to landscape under conditions of varying directional and spectral quality.

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TABLES

Table 3.1 Leaf morphology differences between plants grown with and without supplemental light.

	Leaf Thickness	Palisade Thickness	Mesophyll Thickness
H. annuus +	288.2 *	161.3 *	112.9
H. annuus -	225.8	92.3	106.4
A. retroflexus +	206.1	52.1 *	109.1 *
A. retroflexus -	179.3	46.7	80.2

+ Plants grown with supplemental lighting, - Plants grown without supplemental lighting, *: $p < 0.05$ for paired t-test, all values are in µm.

FIGURES

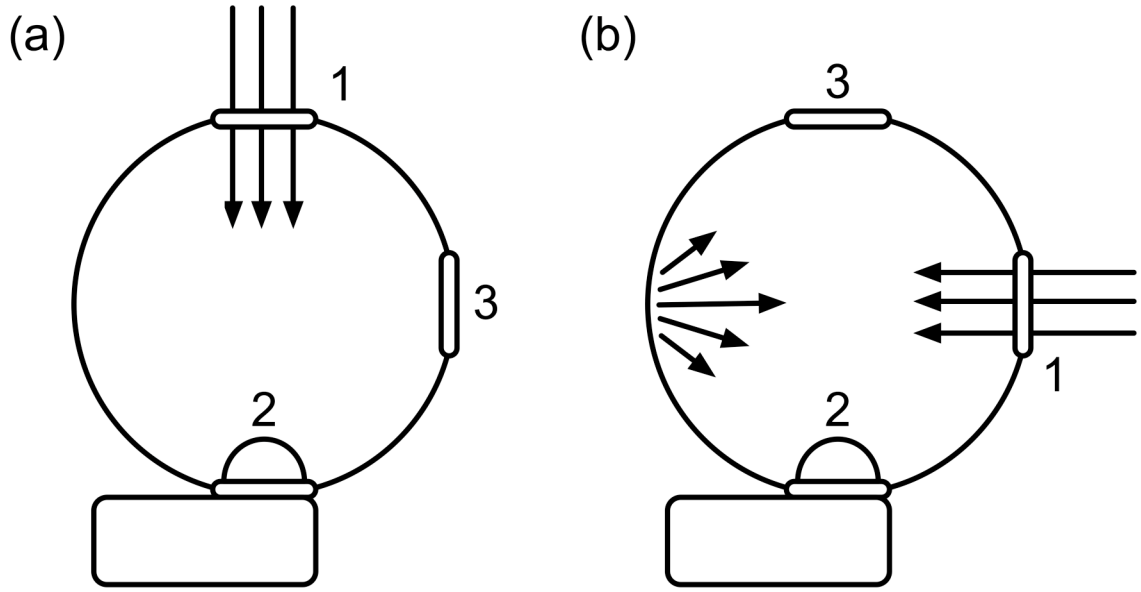

Figure 3.1 Configuration of light source and an integrating sphere to irradiate leaves with direct or diffuse light for photosynthesis. (a) Direct light consisted of a collimated beam that entered an integrating sphere through an open port (1) and passed directly through the sphere to the chamber head (2) of a LI-6400 (NB Li-Cor Inc., Lincoln, NE, USA) where a leaf was mounted perpendicular to the beam. (b) Diffuse light was created by directing collimated light through a port (1) on the equator of the sphere where it struck the interior wall and then was multiply scattered within the sphere to create a diffuse radiation field on a leaf in the LI-6400 chamber (2). Ports (3) were closed with reflective covers when not in use.

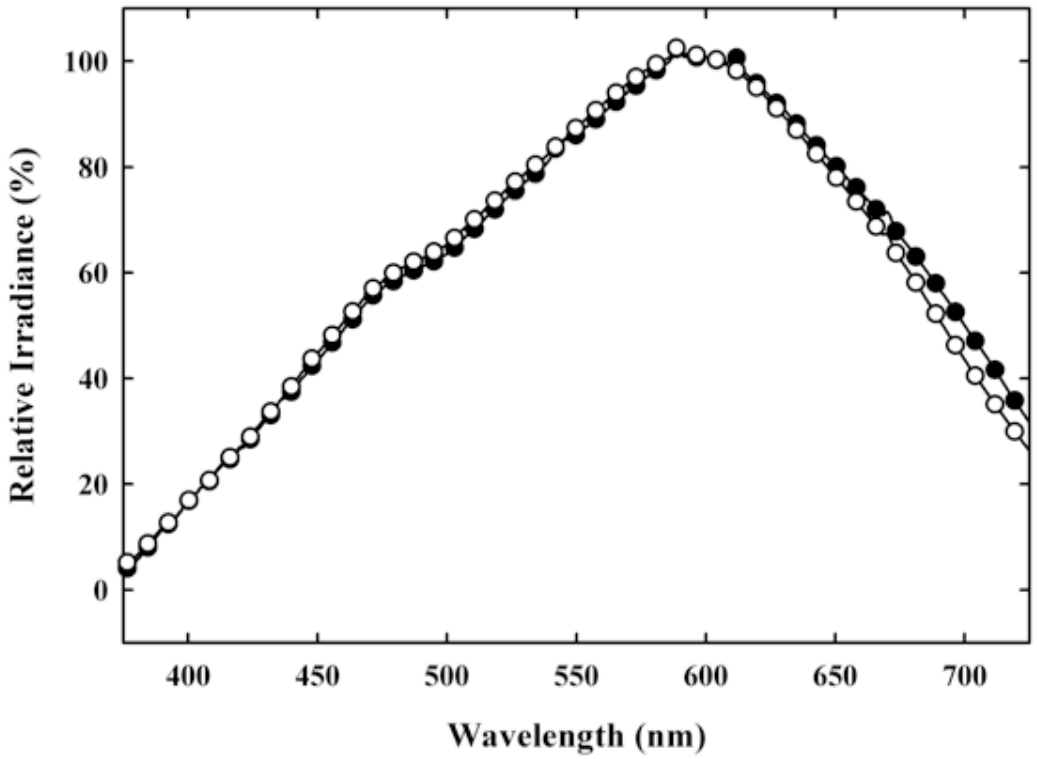

Figure 3.2 Spectral analysis of the direct (closed symbols) and diffuse (open symbols) light sources used in the photosynthetic light response measurements. Spectra were normalized at 605 nm.

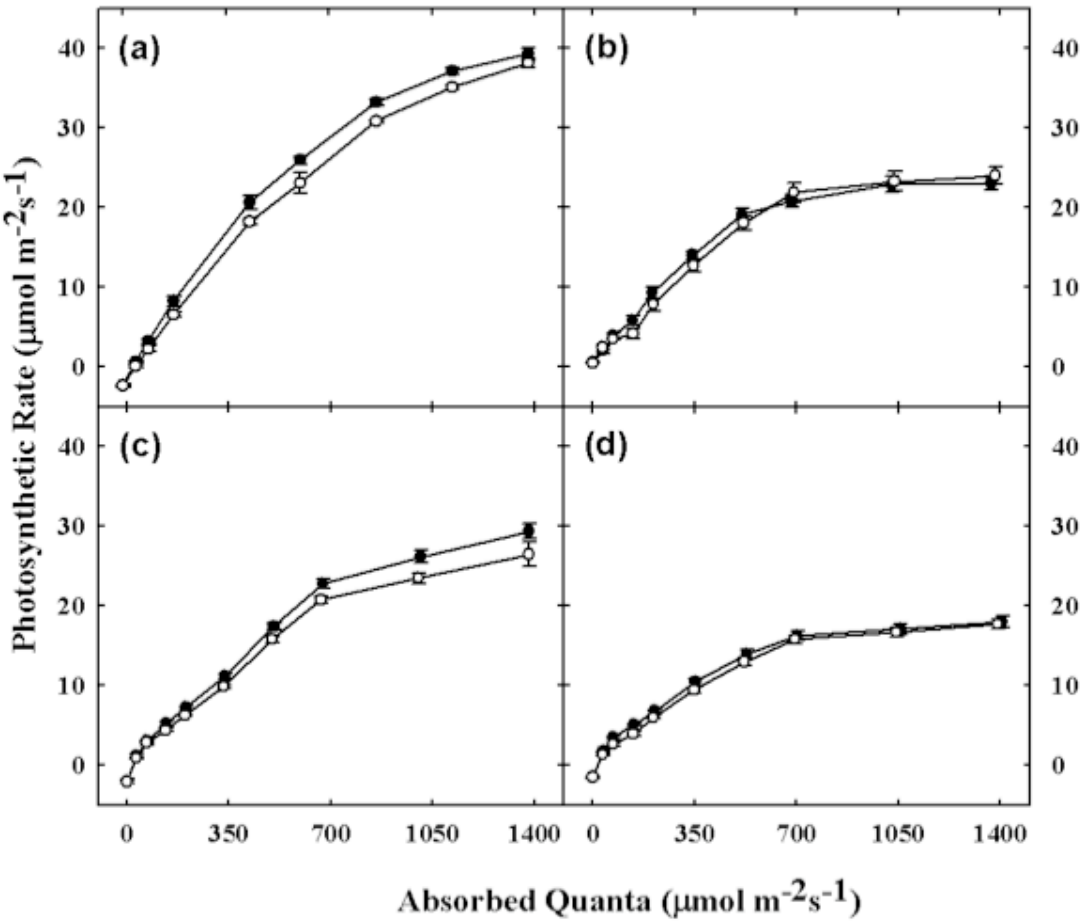

Figure 3.3 Photosynthetic response to direct and diffuse light for (a) *Helianthus annuus* under direct (closed symbols) and diffuse (open symbols) light grown with supplemental lighting; (b) *H. annuus* under direct (closed symbols) and diffuse (open symbols) light grown without supplemental lighting; (c) *Amaranthus retroflexus* under direct (closed symbols) and diffuse (open symbols) light grown with supplemental lighting; (d) *A. retroflexus* under direct (closed symbols) and diffuse (open symbols) light grown without supplemental lighting. All error bars – SE, *n* = 6.

CHAPTER 4: LIGHT ABSORPTION PROFILES UNDER DIRECT AND DIFFUSE LIGHT; EFFECTS OF ANATOMY ON LEAF OPTICS

DO DIRECTIONAL CHANGES IN LIGHT AFFECT ABSORPTION PROFILES?

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ABSTRACT

Absorption profiles of direct, diffuse and low-angle light were estimated using chlorophyll fluorescence imaging coupled with integrating sphere technology. Absorption profiles for direct light were similar in shape to previously published profiles. Overall, diffuse and low-angle light did not penetrate as deep into leaf tissue as direct light, and the greatest differences were observed at the interface between the palisade and spongy mesophyll tissue. Diffuse and low-angle light penetration were similar, and were absorbed close to the illuminated surface while the attenuation of direct light did not decrease as rapidly. Maximum absorption of diffuse light shifted toward the illuminated surface, suggesting a potential mismatch between light absorption profiles and photosynthetic capacity in leaves adapted to intense, collimated light. These data suggest a mechanism that may help explain decreased leaf-level photosynthetic rates under diffuse light.

INTRODUCTION

In nature, light consists of a mix of direct light, where the rays are parallel, and diffuse light where the direction of travel is randomized. On a clear day, the solar radiation that arrives at the Earth's surface consists of 85% direct light and 15% diffuse skylight, but the diffuse component can increase significantly by the presence of clouds and by leaf canopies that scatter the light (Bird & Riordan 1986). During the course of a day, leaves can be irradiated with direct light, which can strike from any number of directions, and diffuse light; and the mix can vary greatly depending on solar elevation, vegetation and weather.

Changing light direction or altering the directional quality of the light can have different effects on photosynthesis. Early work (Yates 1981) showed that leaves respond photosynthetically much like cosine sensors when direct light intercepts the leaf blade from different directions. Maximum rates of photosynthesis were measured when light was directed to the leaf perpendicular to the surface, and photosynthesis declined as the light beam was moved such that it struck the leaf from more oblique directions. Based on these studies, it is generally thought that the photosynthetic rate of leaves is proportional to the amount of light that they absorb, and that there are no other effects of altering light direction on the photosynthetic performance of leaves.

Measurements of the photosynthetic response of leaves to light of different directional quality are more recent. In plants grown under high light, photosynthesis was

15% higher when leaves were irradiated with direct light compared to equivalent absorbed amounts of diffuse light (Brodersen & Vogelmann 2008). While sun leaves perform better photosynthetically under direct light, shade leaves showed no preference for direct or diffuse light, suggesting that leaf anatomy or biochemistry that develops during leaf growth may be responsible for the differential response to direct and diffuse light. Differences in absorption of direct and diffuse light are relatively small and leaf absorptance is 2-3% higher under direct light (Brodersen & Vogelmann 2007). Correcting the photosynthesis measurements for light absorption indicates that the difference in photosynthetic performance is caused by something else (Brodersen and Vogelmann 2008; 2007). One possibility is that, although total leaf absorptance of direct and diffuse light may be similar, the internal distribution of absorbed light within the leaf may be different under the two contrasting light regimes. Given that the photosynthetic performance of cell layers within leaves is a product of their photosynthetic capacity and the amount of light that they absorb, altering internal profiles of absorbed light could alter photosynthetic performance at the whole leaf level (Evans & Vogelmann 2003, 2006).

Chlorophyll fluorescence can be used to measure the distribution of light absorption within the tissues of a leaf (Takahashi et al. 1994; Koizumi et al. 1998). In this method, monochromatic light is directed at the adaxial surface of a leaf sample that is mounted vertically on the stage of a microscope. The cross-sectional view of the leaf can be revealed by making a transverse cut, and the leaf tissues viewed with the microscope. The monochromatic light that passes into the leaf tissue stimulates chlorophyll fluorescence, which escapes from the cross sectional view and is captured with a CCD camera. Since fluorescence is proportional to the amount of light absorbed, images of

chlorophyll fluorescence from the cross sectional view of the leaf reveals the internal light absorption profile (Takahashi et al. 1994; Koizumi et al. 1998; Vogelmann & Han 2000; Johnson et al. 2005; Evans & Vogelmann 2006).

In spinach and eucalyptus leaves, the shapes of light absorption profiles were determined by the wavelength of light, tissue anatomy, and the amount of pigmentation (Evans & Vogelmann 2006). Wavelengths that were strongly absorbed, such as those within the red and blue, created absorption profiles that declined exponentially with increasing depth within the leaf. Blue light is absorbed most strongly and is usually absorbed almost completely by the first few cell layers adjacent to the irradiated surface. Green light penetrated deeper into leaves and was absorbed more equally throughout the mesophyll. Absorption profiles extended further into the palisade than the spongy mesophyll, suggesting that the columnar palisade cells facilitate penetration of light to photosynthetic tissues deep within the leaf. All of these measurements were made with direct light that irradiated the leaf surface perpendicularly. It is not known whether absorption profiles change shape when the beam of direct light intercepts the leaf at angles other than perpendicular, or whether absorption profiles are the same under direct and diffuse light.

It would be surprising if light absorption profiles were insensitive to changes in light direction or directional quality. Given the relatively large change in refractive index between air (n = 1) and the leaf surface (n = 1.42), Snell's Law predicts that a ray of light that strikes the leaf obliquely would change its direction of travel as it enters a leaf. Light scattering by the intercellular air spaces confounds modeling of light propagation within leaves (Vogelmann 1993; Richter & Fukshansky 1996) and, given the additional

complication that leaf tissues consist of cells of different size and shape, it is difficult to predict how light absorption profiles could be altered by changes in light direction and directional quality.

The purpose of the present study is to determine the extent to which absorption profiles within leaves are affected by changes in the directional quality of the incident light, and to determine whether they change in response to changing the angle of incidence of a direct beam of light. The goals are to assess whether changes in light absorption profiles could explain the measured differential photosynthetic response of leaves irradiated with direct and diffuse light and to elucidate whether changes in light direction in the environment could affect the photosynthetic performance of leaves.

METHODS

Helianthus annuus (L.) and *Antirrhínum majus* (L.) were grown in a greenhouse under two conditions. One group of plants was grown under 500 μmol $m^{-2}s^{-1}$ (high-light) provided by daylight supplemented with lamps (400W Phillips ED-18 HPS lamps, Phillips Inc.) and a second group was grown without supplemental lighting where the irradiance was 150 μmol $m^{-2}s^{-1}$ PPFD (low-light). Hereafter, leaves grown under high-light and low-light will be referred to as sun and shade leaves respectively. Average daytime temperature was 30˚C.

Absorption profiles in leaves were measured in a manner similar to that described previously (Takahashi et al. 1994; Koizumi et al. 1998; Vogelmann & Han 2000;

Vogelmann & Evans 2002). Leaf samples were placed on a holder on the stage of a microscope (Olympus BX60, Olympus America, Inc., Center Valley, Pennsylvania) and were irradiated from one side (Fig. 4.1, 4.2) in sequence with monochromatic red (660 nm), green (532 nm), or blue (488 nm) light obtained from one of three lasers (red solid state laser: Model #BWN-660-10E, B&W Tek Inc., Newark, Delaware; green solid state laser: Model # DY20B, Power Technology Inc., Little Rock, Arkansas; and blue argon gas laser: Model # Innova 300, Coherent Inc., Santa Clara, California). Light from the blue argon laser passed through a 488 nm laser line filter (#FL488-10, CWL = 488 ±2 nm, Thorlabs, Inc., Newton, New Jersey) to exclude extraneous wavebands. Light from the lasers was attenuated by passing the beam through a 0.3 O.D. metallic neutral density filter (Melles Griot, Inc., Rochester, New York) and then a lens (25.4 mm diameter Plano Convex Lens, focal length = 75.0mm, #BPX065, Thorlabs, Inc., Newton, New Jersey) to spread the beam into a 6 mm spot on the leaf surface (Fig. 4.1a). Irradiance values for each light source and wavelength were measured by mounting an LI-190 quantum sensor in the same position normally occupied by the leaf sample, and values averaged 415, 400, and 550 μmol $m^{-2}s^{-1}$ PPFD for direct red, green, and blue light respectively, and 150, 330, and 250 μmol $m^{-2}s^{-1}$ PPFD for diffuse red, green, and blue light respectively. Leaves were also illuminated with direct red, green and blue light on a rotating stage, allowing measurement of chlorophyll fluorescence in leaves irradiated at 0˚ (perpendicular to the leaf surface), 30˚, and 60˚ (Fig 4.1a).

For diffuse light measurements, the laser light was directed with a series of mirrors into a port (0.4 mm diameter opening) of a 2.5 cm diameter custom integrating sphere 90˚ from the exiting light port (6 mm x 8 mm opening), from which diffuse light

illuminated the leaf surface (Figs. 4.1b, 4.2b). Leaves were also assessed for chlorophyll distribution by irradiating the cut surface via epi-illumination (490 nm).

Images of chlorophyll fluorescence passed through a barrier filter (680 nm, half band width = 16 nm, S10-680F; Corion Filters, Franklin, Massachusetts) and were captured with a digital camera (PIXIS 1024B, Princeton Instruments, Trenton, New Jersey). Images were processed using Image Pro Plus 6.0 software (Media Cybernetics, Silver Spring, Maryland). Statistical calculations and graphing were performed using Sigma Plot 9.0 (Systat Software, San Jose, California).

RESULTS

Leaf anatomy

The two species in this study had different developmental responses to the low and high light environments, producing leaves that differed in thickness and tissue composition. *H. annuus* leaves grown under sun and shade conditions were very similar in thickness (Table 1), but the amount of palisade and spongy mesophyll varied. In shade leaves, there were similar amounts of palisade and spongy mesophyll tissue (ratio of 1:0.96) whereas there was more palisade in sun leaves (1:0.71). In *A. majus,* the palisade to spongy mesophyll ratios of sun and shade leaves were similar (1:0.71 and 1:0.83 respectively), but there were greater differences in leaf thicknesses. Sun leaves of *A. majus* were 23% thicker than shade leaves, while *H. annuus* sun leaves were only 4% thicker than shade leaves.

Light absorption profiles in leaves irradiated at different angles of incidence

The extent to which light penetrated into a leaf and was absorbed within the photosynthetic tissues was shown by chlorophyll fluorescence profiles. Upon entering a leaf, blue and red light were absorbed strongly by the mesophyll and 80% of the light was absorbed within the initial 100 μm of tissue (Fig. 4.3a,c). Green light penetrated deeper within the leaf and was distributed more uniformly to the tissues (Fig. 4.3b). When the angle of incidence changed from perpendicular (0°) to more oblique directions (30°, 60°), the absorption profiles became compressed and tissues adjacent to the irradiated surface absorbed more of the light. The largest effects were found under green light (Fig. 4.3b) where both the depth of light penetration and the shape of the absorption profile changed with angle of incidence. When irradiated at 0°, the amount of chlorophyll fluorescence rose gradually with increasing depth within the leaf, reaching a maximum at 200 μm, and falling off linearly thereafter. Changing the angle of incidence to 60° caused a marked change in the shape of the absorption profile where the chlorophyll fluorescence maximum shifted to 75 μm depth, and then declined linearly thereafter. When leaves were irradiated with blue or red light, the light absorption profiles decreased in an exponential manner with increasing depth and the shapes did not change appreciably with different angles of incidence. The smallest effects occurred under blue light, which was absorbed more intensely than green or red light.

Light absorption profiles in leaves irradiated with direct and diffuse light

Images of chlorophyll fluorescence show striking differences when leaves were irradiated with direct vs. diffuse light (Fig. 4.4). Within the blue, red and green regions of the spectrum, chlorophyll fluorescence showed that direct light penetrated further into the leaf than diffuse light. In addition to differences in depth of penetration, there were other qualitative differences in the images of chlorophyll fluorescence. Images obtained when leaves were irradiated with direct light were crisper and the cellular features were more clearly defined compared to leaf samples irradiated with diffuse light. For example, under blue light, the cell walls are more clearly visible in samples irradiated with direct light (Fig. 4.4e) compared to diffuse light (Fig. 4.4f). This may indicate that there is a relationship between the direction that fluorescence is emitted and propagated within a leaf and the direction that light is intercepted and absorbed by chloroplasts.

Quantifying light absorption profiles through image analysis showed that the directional quality of the incident light and leaf anatomy had marked effects on the characteristics of absorption profiles within leaves, influencing the shape of the profiles and the depth to which light penetrated. In all cases examined, direct light penetrated further into leaves than diffuse light, and the largest effects were observed in the green as opposed to the blue and red.

Measuring light absorption profiles in *A. majus* showed that they had similar shapes in sun and shade leaves (Figs. 4.5, 4.6). A notable difference was that the increased palisade in sun leaves (92 μm thicker) allowed greater penetration of red (Figs. 4.5a, 4.6a) and green light (Figs. 4.5b, 4.6b) but not blue light. Palisade tissue facilitated

the penetration of both direct and diffuse light, by ca. 50 μm in the red and 100 μm in the green. When the abaxial surface of the leaf was irradiated, absorption profiles were more compressed in the spongy mesophyll than when light entered the palisade from the adaxial side of the leaf. Light also penetrated further into the spongy mesophyll of sun leaves compared to shade leaves but less so compared to palisade tissue. Although direct light consistently penetrated further into leaves than diffuse light by about 30 μm, this increment was relatively constant irrespective of wavelength and tissue type (Figs 4.5, 4.6).

In *H. annuus* where sun and shade leaves showed larger differences in mesophyll anatomy and where leaf thickness was relatively constant, the absorption profiles had notably different shapes in the two types of leaves (Figs. 4.7, 4.8). Absorption peaks in sun leaves (Fig. 4.7) occurred within the initial 50 μm of the mesophyll and light absorption usually declined exponentially thereafter. The shapes of the absorption profiles were similar when leaves were irradiated adaxially and abaxially with red (Figs. 4.7a, d) or blue light (Figs. 4.7c, f) but not green light. Any of the slight increases in chlorophyll fluorescence in the tail end of these curves are due to slight sampling errors, where the cut surface of the leaf was not perfectly level. Some of the leaf tissue was raised above the focal plane of rest of the surface, causing reflections that not would normally not be observed. The majority of the images where this was grossly evident were not used for this study, but images where the effect was minimal and did not influence the overall results were included (Figs. 4.4c, 4.5f, 4.7f).

Epi-illumination and Chlorophyll Distribution

Chlorophyll distribution within leaf tissues was approximated by observing the relative chlorophyll fluorescence profiles induced by epi-illumination (Fig. 4.9). The differences in relative chlorophyll fluorescence within *H. annuus* (Fig. 4.9b) sun and shade leaves were greater than in *A. majus* (Fig. 4.9a). Shade leaves of *H. annuus* showed higher chlorophyll fluorescence throughout the first 60% of the leaf thickness, with up to 15% difference between sun and shade leaves. Peak relative chlorophyll fluorescence in both sun and shade *H. annuus* leaves occurred near the abaxial surface. In *A. majus* sun leaves, chlorophyll fluorescence peaked near the palisade-spongy mesophyll transition, while fluorescence in shade plants was shifted toward the abaxial surface. Variability in relative chlorophyll fluorescence between sun and shade leaf samples of *A. majus* was relatively low for the first 80% of the leaf tissue, but variability increased toward the abaxial surface (Fig. 4.9a). In *H. annuus* the variability in relative chlorophyll fluorescence between samples was reduced when compared to *A. majus* throughout the leaf, although variability increased near the abaxial surface (Fig. 4.9b).

DISCUSSION

The light environment of plants is complex and is composed of strong gradients in light intensity, changes in light directionality, and altered spectral quality (Niinemets 2007). Leaves can be irradiated with direct, diffuse and low-angle light, but the photosynthetic effects of changes in light directionality have only recently been shown to

be important. Acclimation to a variable light regime is a hallmark of morphological plasticity that results in sun and shade leaf adaptations that maximize light use efficiency. This study allowed for the first time the comparison of direct, diffuse and low-angle light absorption profiles in sun and shade leaves of two broadleaf species.

Contrary to our expectations, total leaf thickness in sun and shade *H. annuus* leaves were not significantly different ($p > 0.05$) and light intensity in the sun treatment may not have been high enough to stimulate the development of thicker leaves characteristic of this species. However, the light conditions were adequate to produce sun and shade phenotypes in *A. majus*, where sun leaves developed thicker palisade tissue, while shade leaves developed thicker spongy mesophyll. Sun and shade leaves of *A. majus* had similar palisade to spongy mesophyll tissue ratios (1:071 and 1:0.83, respectively). Differences in total leaf thickness in sun and shade *H. annuus* leaves were not significant, but palisade tissue was thicker in sun leaves and spongy mesophyll was thicker in shade leaves. Despite the lack of morphological differentiation, the growth conditions were identical to those used by Brodersen et *al*. (2008) which yielded *H. annuus* sun plants that showed a 15% increase in photosynthesis under direct vs. diffuse light.

Absorption profiles in *A. majus* showed that low-angle direct light (30˚ and 60˚) did not penetrate as deeply into the leaf tissue as light that struck the leaf perpendicularly (0˚). When leaves were irradiated obliquely, light absorption for low-angle light was shifted toward the illuminated surface, and this was most evident under green light. This shift in the absorption profile illustrates how light distribution changes within a leaf in the natural environment when the solar elevation is low. Light entering a canopy late in the

day will intercept a horizontal leaf at similar low angles and internal light absorption will shift towards the irradiated surface, concentrating light absorption within these tissues. The orientation and display angle of leaves in the canopy will ultimately determine the amount of light intercepted by leaves (Ehleringer & Werk 1986; Smith et al. 1997; Smith et al. 1998). Perfectly horizontal leaves are not common in nature, and the majority of leaves are angled to maximize light interception. Because low-angle light will not penetrate as deeply, the benefits of fine-tuning leaf orientation are clear.

The shape of low-angle and diffuse light absorption profiles were similar, although low-angle light profiles were more compressed. When compared to direct light both diffuse, 30˚ and 60˚ light attenuation was stronger throughout the thickness (Figs. 4.4, 4.5). Because diffuse light is composed of a small amount of direct beam radiation, we would expect low-angle light to produce a more shallow absorption profile.

There was a remarkable change in the penetration of green light between absorption profiles in sun and shade leaves. These data indicate that under diffuse light more light will be absorbed within the tissue adjacent the surface compared to leaves irradiated with direct light. The shift in green light absorption peaks did not occur when leaves were irradiated abaxially, suggesting that palisade and spongy mesophyll tissue interact with diffuse light in different ways (Fig. 4.5e). Chlorophyll distribution could also play a role in determining the shape of the absorption profiles. Maximum fluorescence from epi-illumination occurred near the abaxial surface, and abaxial absorption profiles may have been masked by strong absorption in that tissue.

Palisade tissue appeared to influence the passage of light through leaves, and this is particularly evident under green light (both diffuse and low-angle). Because red and

blue light are strongly absorbed within the first 20% of the leaf tissue, under either adaxial or abaxial illumination, the effects of the palisade tissue on light absorption properties are not easily observable. Both direct and diffuse green light penetrated much deeper into the leaf tissue sun in *A. majus* leaves that had better defined palisade tissue. Palisade tissue appeared to function as light pipes, channeling light to deeper tissue depths as previously suggested by various studies (Vogelmann & Evans 2002; Vogelmann 1993; Terashima & Saeki 1983). This was only observable when light entered through the adaxial surface, and was evident under green light and to a lesser degree in red light.

With proportionally more light absorbed at the adaxial surface, shade leaves may be better suited to take advantage of low intensity diffuse green light that would be prevalent in the understory. The presence of thin palisade tissue and thick spongy mesophyll in shade leaves could aid in the absorption of green light within the upper tissue layers. Backscattering of light within the palisade tissue and from the upper spongy mesophyll may direct photons back to the upper tissue layers. Thus, the increase in path length of light within the leaf increases the likelihood of light interception and absorption by a chloroplast (DeLucia et al. 1996). Under diffuse light, photons entering at low angles relative to the leaf surface have a greater probability of coming into contact chloroplasts lining the periclinal walls of the palisade tissue. This is because low-angle (e.g. 60˚) light must travel through more leaf tissue than light entering perpendicular to the leaf (0˚). As the angle of incidence increases (moves away from perpendicular), the probability of photons intersecting multiple cell walls to intercellular air space interfaces

increases. However, light passing through two substances of different refractive index will change direction, causing low-angle or diffuse light to become more collimated.

Light entry into leaves has been shown to depend on the surface qualities of the epidermis. Brodersen & Vogelmann (2007) found that plants absorb more light when it is direct than when diffuse, primarily due to greater reflectance of diffuse light. Epidermal lens cells have been shown to focus direct light, leading to light intensities several fold greater than incident light beneath the cells. They do not, however, appear to aid in the absorption of diffuse light (Vogelmann et al. 1996; Brodersen & Vogelmann 2007). Similarly, Govaerts et al. (1996) modeled the fate of photons originating from various illumination angles, and found that with increasing angle of incidence, more light is lost to specular, mirror-like reflection and less light is transmitted into the leaf. Thus the epidermis plays a role in light absorption.

Leaf orientation may be even more critical in whole-crown and leaf-level absorption (Schymanski et al. 2007). Falster and Westoby (2003) suggest that orienting leaves at steeper angles in some tree species may have evolved to enhance scattering of incoming direct sunlight and increase the diffusion of light into the canopy, thereby increasing the total leaf area illuminated within the canopy. This idea is corroborated by community-level studies that observed greater primary productivity of forests under diffuse light, where the whole canopy is more evenly illuminated (Norman, 1971; Roderick et al., 2001; Farquhar et al., 2003; Gu et al., 2003). Here we show that in the outermost leaves of a canopy steeper leaf angles will orient the leaf so it is more perpendicular to the sun, thereby increasing the penetration of light into the leaf tissue.

Snell's Law describes how light propagation within a transparent material is

determined by refractive index and the angle that light intercepts the surface (Richter & Fukshansky 1996; Vogelmann 1993). Changes in the angular distribution of light incident at the leaf surface could alter the path of light through leaves, and direct and diffuse light may travel differently through the tissue of the same leaf. Leaf development under sun (more direct light) and shade (more diffuse light) conditions establishes chloroplast and Rubisco distributions to maximize light harvesting efficiency within the opposing gradients of light and CO_2 (Smith et al. 1997; Evans 1999; Terashima et al. 2006). If light propagation through leaves is sensitive to the angle of incidence then the internal light absorption profile and photosynthetic capacity of leaves may become uncoupled, thereby decreasing the ability of leaves to utilize light efficiently. Photosynthesis would then be reduced when leaves adapted for environments with high light intensity and greater proportions of direct light are irradiated with diffuse light.

The difference in absorption profiles for sun leaves is likely a key mechanism in explaining differences in leaf-level photosynthesis under direct and diffuse light. To evaluate the mechanism responsible for the different photosynthetic responses to direct and diffuse light it is necessary to both (1) identify where light is absorbed in the leaf when illuminated with direct and diffuse light and (2) determine the distribution of photosynthetic capacity in the leaves. The optimal overlap between (1) and (2) could lead to maximum photosynthetic performance. Any mismatch between those two functions could lead to less efficient light utilization (Evans & Vogelmann 2006), and possibly the differences in leaf-level photosynthetic performance observed by Brodersen & Vogelmann (2008). Thus, if direct and diffuse light penetrate though leaves differently, yielding different light absorption profiles, leaves in a high light environment

that have adapted their internal leaf anatomy and chlorophyll distribution for utilizing direct light efficiently may not be able to optimally utilize diffuse light.

This study clearly shows that the passage of light through leaves is more complex than once believed, and leaves are indeed sensitive to the directionality of light. Because diffuse and low-angle light does not penetrate as deeply into leaves, likely due to increased scattering and path-lengthening, the directionality of light may be one of the many signals that determine leaf anatomy (Terashima et al. 2006), giving greater importance to the light environment of developing leaves in maintaining successful light utilization. Most importantly, an optimized overlap between light gradients and photosynthetic capacity is essential in maximizing light utilization. Changes in the directionality of light throughout the day and growing season could alter that optimization in leaves that adapted to an environment with predominantly more direct or diffuse light.

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TABLES

Table 4.1 Leaf anatomy characteristics for *A. majus* and *H. annuus* grown with and without supplemental lighting. P:S = Palisade to Spongy Mesophyll Ratio. * = non-significant ($p > 0.05$).

Species	Light Treatment	Total Leaf Thickness	Palisade Thickness	Mesophyll Thickness	P:S Ratio
A. majus	High Light	547.8	284.1	206.5	1.4
A. majus	Low Light	421.4	191.7	163.7	1.2
H. annuus	High Light	224.6*	117.4	84.8	1.4
H. annuus	Low Light	214.3*	99.1	95.2	1.0

FIGURES

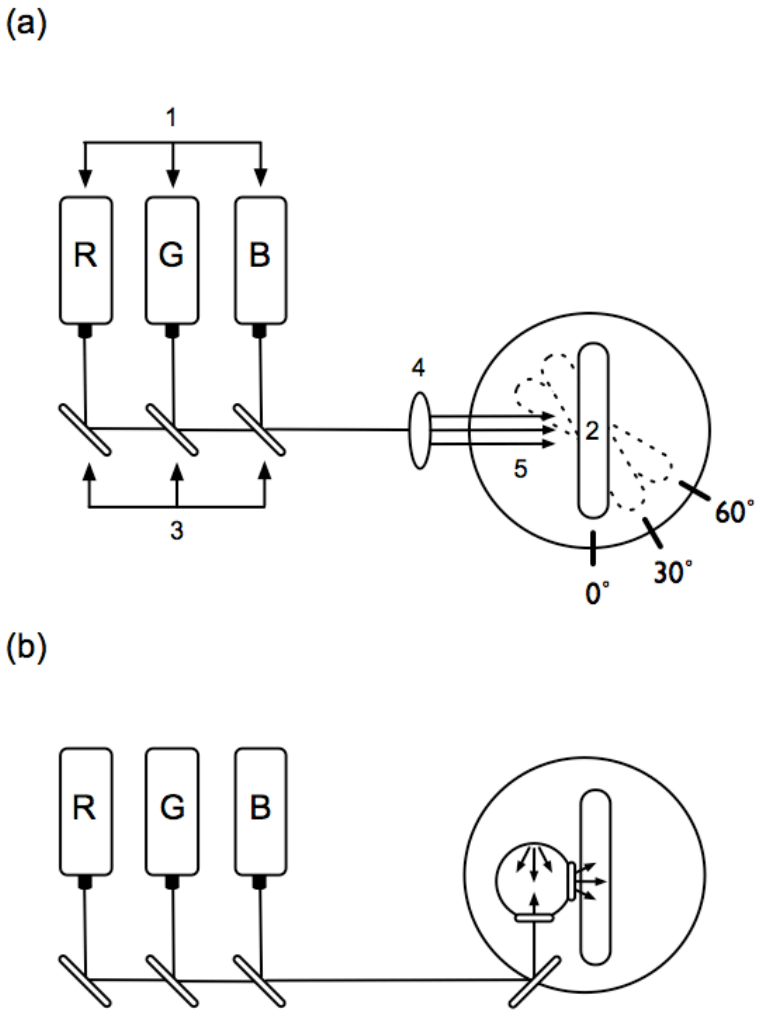

Figure 4.1 Diagram of the direct and diffuse light sources used to irradiate a leaf sample viewed with a microscope. (a) Monochromatic laser light (1) red, green, or blue (660, 532, 488 nm, respectively) was directed towards a leaf sample (2) on a microscope stage by surface silvered mirrors (3). Light from each laser was directed to the sample in sequence by retracting the appropriate mirrors. Light passed through a lens (4) to spread the beam from an initial diameter of 2 mm to 6mm. A neutral density filter (not shown) was used to adjust the irradiance at the leaf surface to 200-400 µmol m-2s-1 PPFD. The leaf sample could be rotated on the microscope stage so the incident light was 0˚, 30˚, or 60˚ with respect to the leaf surface. (b) The direct light from the lasers was made diffuse by inserting an integrating sphere into the light path in which the light entered through a port, was multiply scattered within the sphere, and exited a port adjacent to the leaf surface.

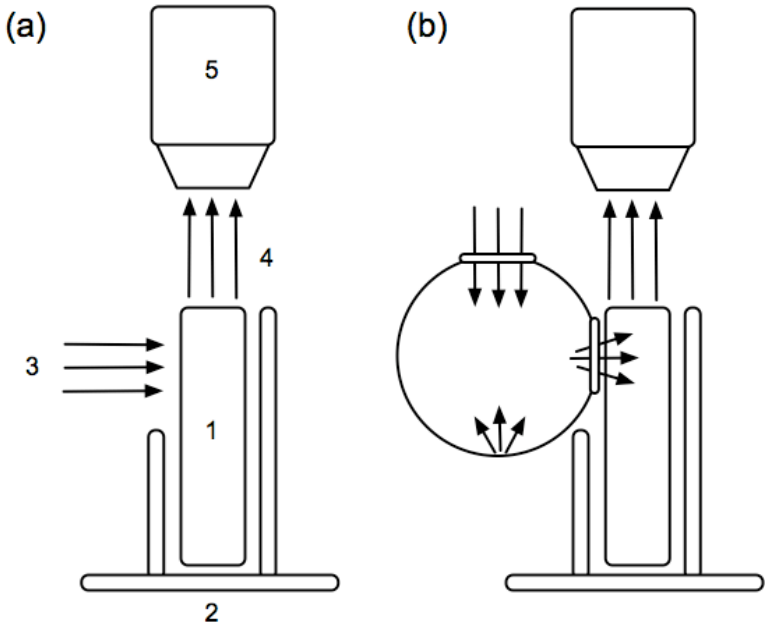

Figure 4.2 Diagram of the leaf holder, light path, and integrating sphere. (a) The leaf sample (1) was held in a glass sample holder (2) and illuminated with collimated monochromatic laser light (3) as described in Fig. 4.1. The light elicits chlorophyll fluorescence, which was viewed from the cut edge (4) of the leaf sample, observed through a microscope (5). An image was captured with a CCD camera and analyzed with image processing software. (b) Diffuse light was made by inserting an integrating sphere into the light path and images of chlorophyll fluorescence were collected and analyzed.

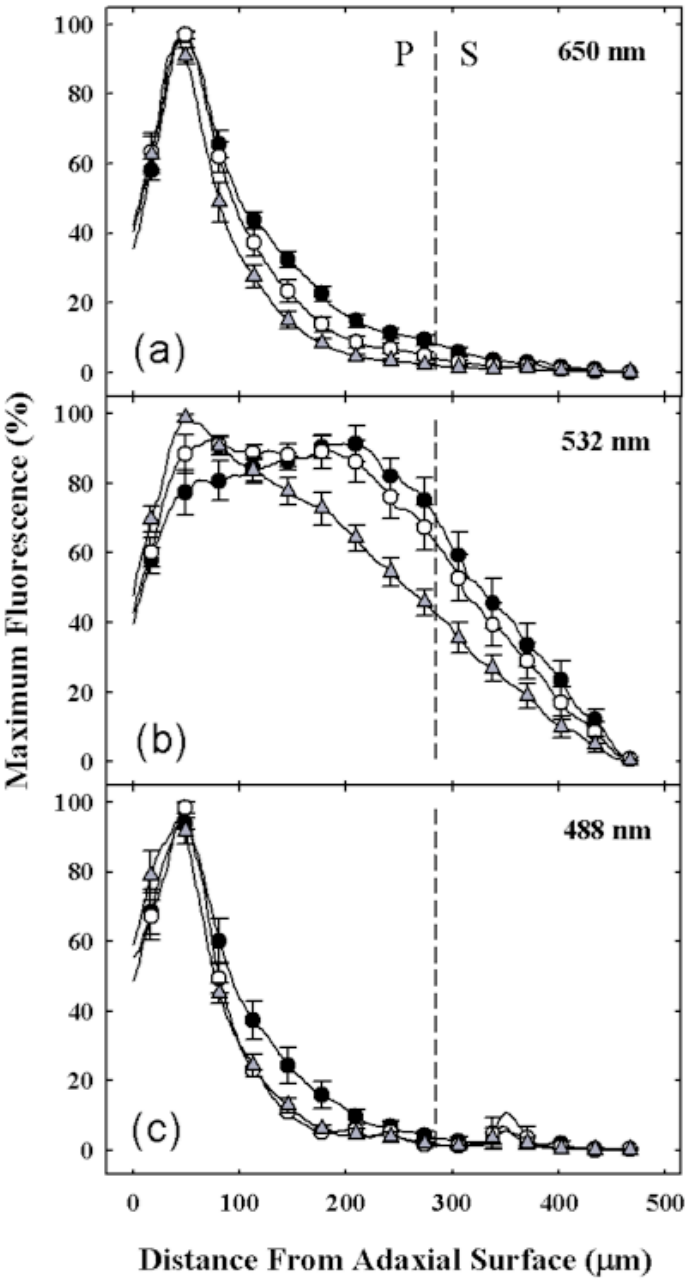

Figure 4.3 Light absorption profiles in sun-adapted leaves of *A. majus* irradiated at different angles of incidence. The adaxial surface was irradiated with red (a), green (b), or blue (c) at 0˚ (perpendicular to the leaf surface, closed circles), 30˚ (open circles), or 60˚ (gray triangles). The vertical dashed line shows the transition between the palisade (P) and spongy (S) mesophyll cell types. Error bars show SE, *n* = 6.

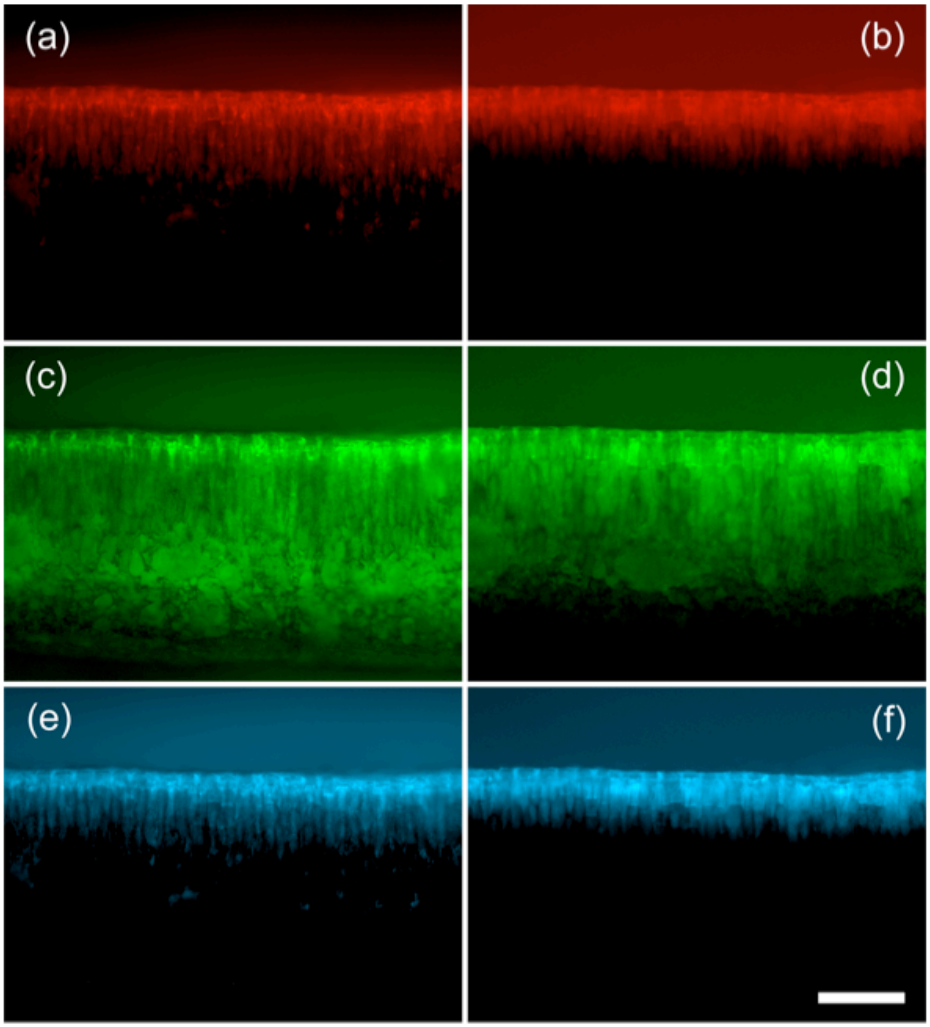

Figure 4.4 Distribution of chlorophyll fluorescence in an *A. majus* leaf irradiated with direct and diffuse light. The leaf was irradiated on its adaxial surface with red (a, b; 650 nm), green (c, d; 532 nm) or blue (e, f; 488 nm) light. Left panels (a, c, e) show chlorophyll fluorescence profiles under direct light, right panels (b, d, f) show profiles in the same leaf irradiated with diffuse light. False color images of fluorescence show profiles of light absorption at the different wavelengths. Scale bar = 250 µm.

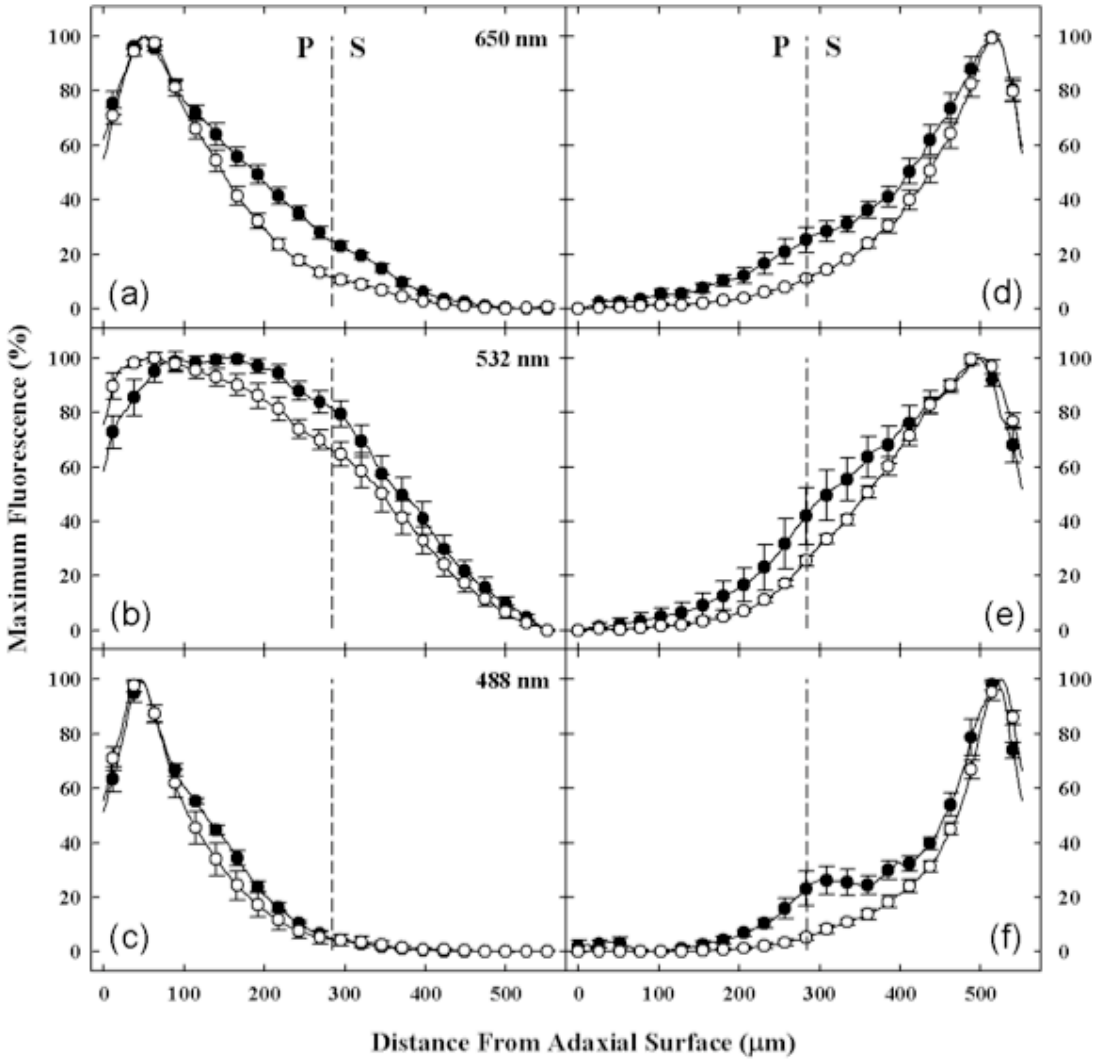

Figure 4.5 Chlorophyll fluorescence profiles in *A. majus* sun-adapted leaves when irradiated with direct (closed circles) and diffuse light (open circles). Leaves were irradiated on their adaxial (a, b, c) or abaxial (d, e, f) surface by red (a, d), green (b, e), or blue (c, f) light. The vertical dashed line denotes the transition between the palisade (P) and spongy (S) mesophyll cell types. Error bars show SE, *n* = 6.

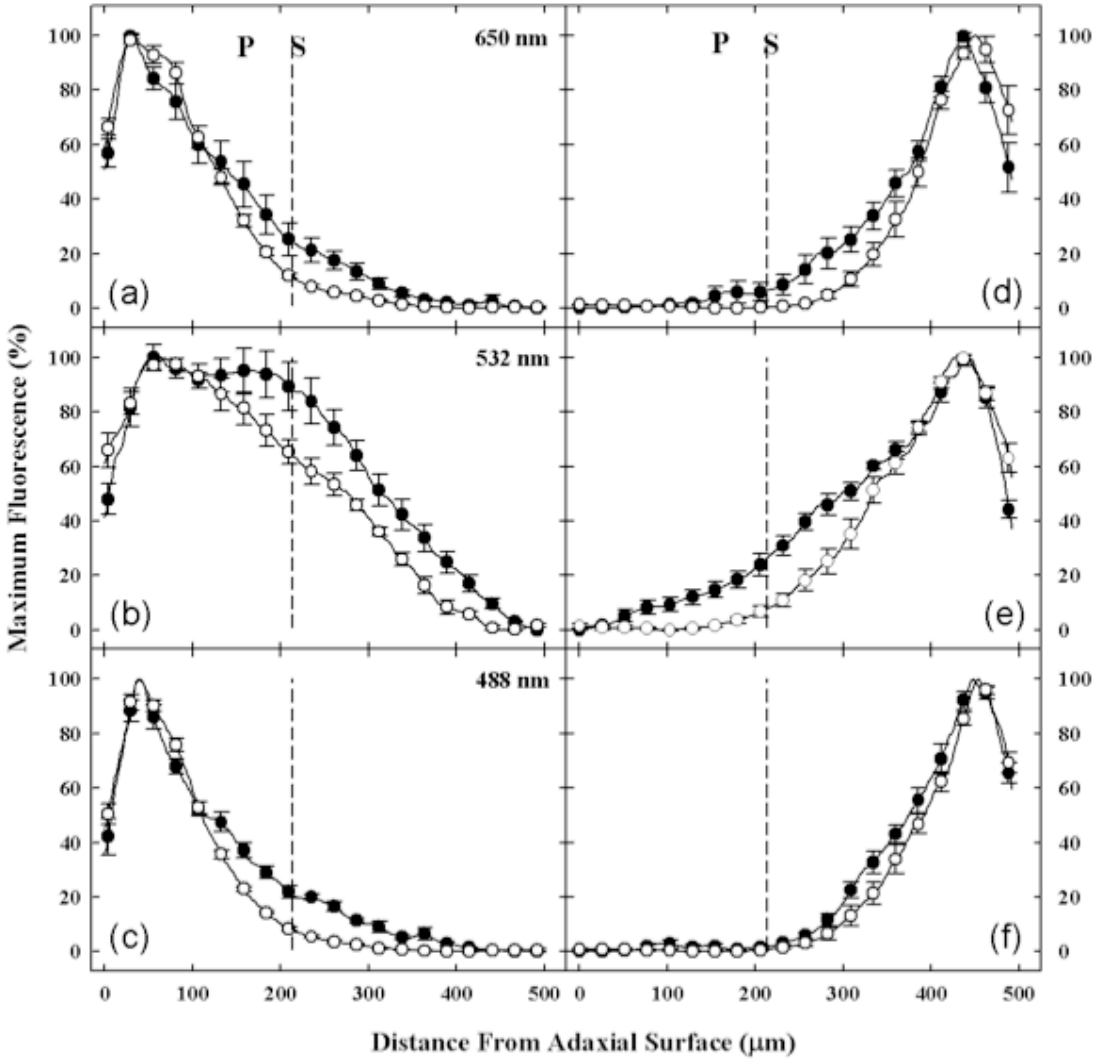

Figure 4.6 Chlorophyll fluorescence profiles in *A. majus* shade-adapted leaves when irradiated with direct (closed circles) or diffuse light (open circles). Leaves were irradiated on the adaxial (a, b, c) or abaxial (d, e, f) surface by red (a, d), green (b, e), or blue (c, f) light. The vertical dashed line denotes the transition between the palisade (P) and spongy (S) mesophyll cell types. Error bars show SE, n = 6.

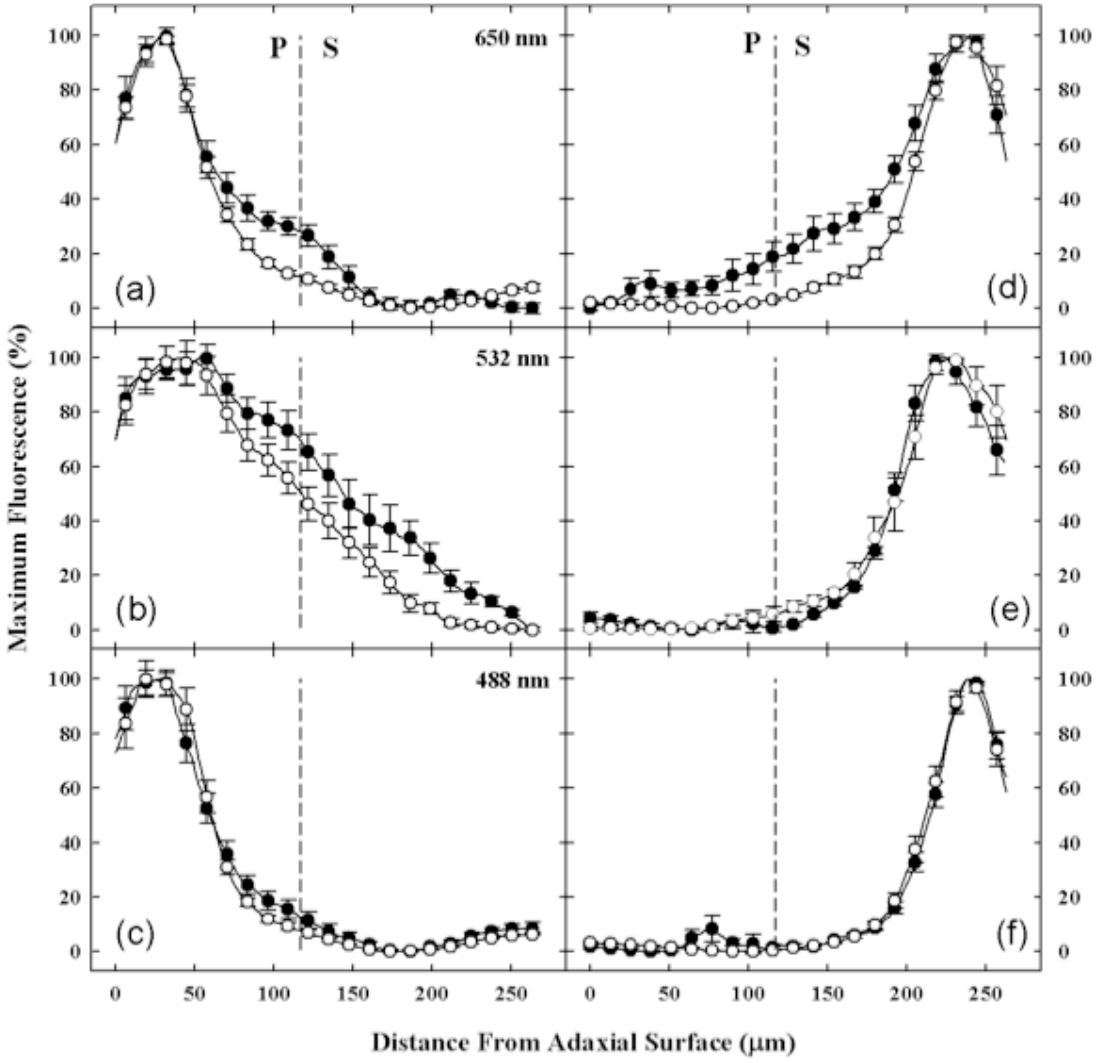

Figure 4.7 Chlorophyll fluorescence profiles for *H. annuus* sun-adapted leaves when irradiated with direct (closed circles) or diffuse light (open circles). Leaves were irradiated on the adaxial (a, b, c) or abaxial (d, e, f) surface by red (a, d), green (b, e), or blue (c, f) light. The vertical dashed line denotes the transition between the palisade (P) and spongy (S) mesophyll cell types. Error bars show SE, *n* = 6.

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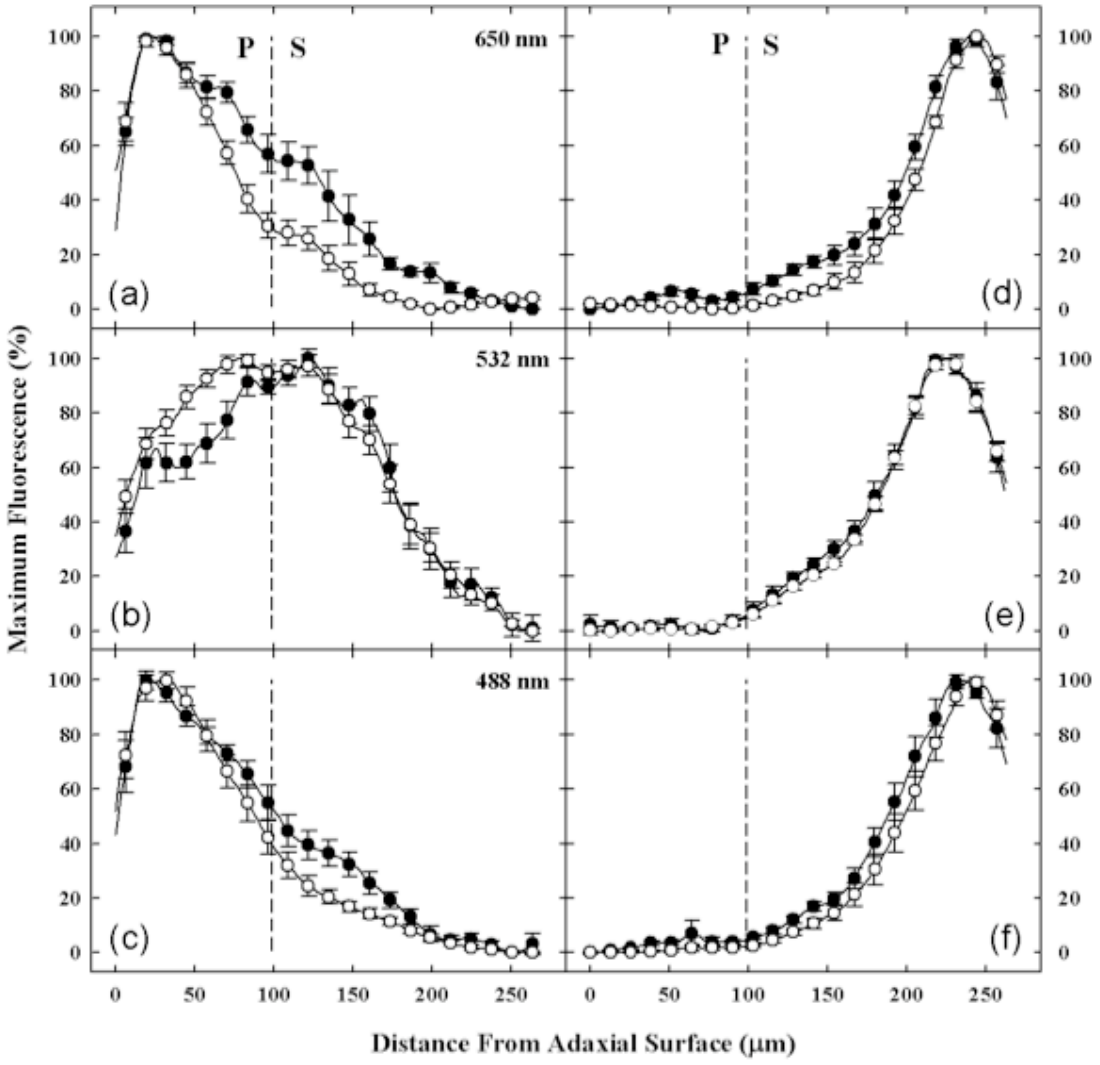

Figure 4.8 Chlorophyll fluorescence profiles in *H. annuus* shade-adapted leaves when irradiated with direct (closed circles) or diffuse light (open circles). Leaves were irradiated on the adaxial (a, b, c) or abaxial (d, e, f) surface by red (a, d), green (b, e), or blue (c, f) light. The vertical dashed line denotes the transition between palisade (P) and spongy (S) mesophyll cell types. Error bars show SE, *n* = 6.

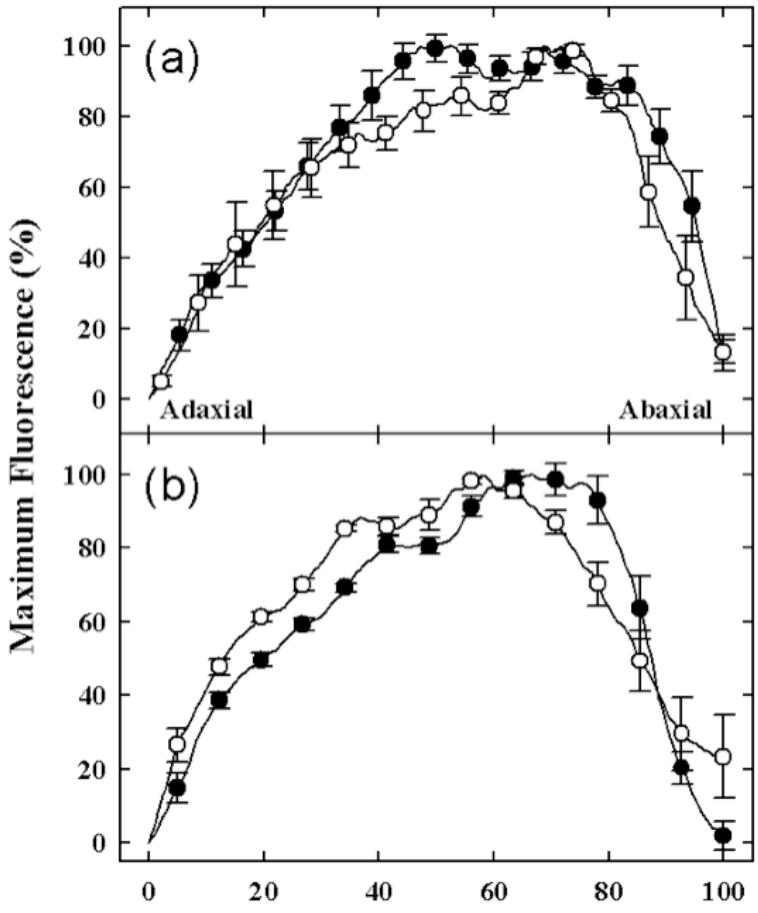

Figure 4.9 Chlorophyll fluorescence profiles in (a) *A. majus* and (b) *H. annuus* sun (closed circles) and shade (open circles) leaves when irradiated with epi-illumination (490 nm). Error bars show SE, n=6.

CHAPTER 5: DISSERTATION SUMMARY

DISCUSSION ON THE IMPLICATIONS OF THIS DISSERTATION

Each of these three experiments provided new insight into the effects of the directionality of light on light absorption and utilization. In each case, these data revealed for the first time that direct and diffuse light are not utilized equally by plants at the leaf level. Most importantly, we now know that leaf architecture has a significant effect on the way that direct and diffuse light are absorbed by leaves.

Current global change scenarios predict increases in cloud cover for many areas on Earth due to increasing water vapor in the atmosphere (Alton et al. 2007; Roderick 2006; Schiermeier 2006). As direct light from the sun passes through clouds and other atmospheric particles it is scattered and becomes diffuse. Recent research has revealed that community-level productivity can be significantly influenced by the directional quality of light. We now know that photosynthesis at the leaf-level is affected by changes in the directionality of light, and the response is opposite to that at the canopy level. Community-level photosynthesis increases by approximately 20% under diffuse light but at the leaf-level photosynthesis decreases by 15% (Alton et al. 2007; Brodersen & Vogelmann 2008; Gu et al. 1999, 2003; Farquhar & Roderick 2003). Expressing photosynthesis on the basis of absorbed quanta, when leaves were irradiated with directional light, they often had photosynthetic rates that were 15% higher then when irradiated with diffuse light. These results may be explained by the finding that light

absorption profiles in leaves are not the same under the two opposing light regimes. Directional light penetrates further into the leaf than diffuse light. The shape of the absorption profiles was determined by the directional quality of the light and leaf anatomy; and light penetrated further through palisade tissue than spongy mesophyll.

The finding that light absorption profiles were determined by the interaction between light directional quality and leaf anatomy, explains our experimental results that showed that the photosynthetic response of leaves to light direction was influenced by their growth environment. Plants in a high light environment utilized direct light much better than diffuse light, while shade-adapted plants of the same species showed no preference for direct or diffuse light. These data suggest that plants change their leaf anatomy and biochemistry to utilize direct or diffuse light most efficiently. While this study showed that sun-adapted broadleaf plants show a preference for direct light, the effects of direct and diffuse light on plants in their natural environment remain unknown.

While community-level studies have included both deciduous and evergreen species, our leaf-level photosynthesis measurements under direct and diffuse light were limited to broadleaf species under laboratory conditions. The light source used for these laboratory photosynthesis experiments is considerably less collimated than natural direct sunlight. Therefore, the differences that we measured for photosynthesis under direct and diffuse light may underestimate that which occurs in plants irradiated with sunlight in their natural environment. Extending the laboratory measurements to the field is necessary to more accurately determine the relationship between light directional quality in the environment and photosynthesis and the impacts of global change on plant productivity.

There is evidence that conifer seedling photosynthesis can be enhanced by diffuse light conditions, primarily due to better light penetration through the canopy and to the forest floor where the seedlings germinate (Johnson & Smith 2006). The leaf, or shoot-level, response to direct and diffuse light in conifers has never been studied, but the orientation of needles on a stem and internal needle anatomy suggest that conifers may benefit from diffuse light more than a broadleaf plant. Under direct light, much of a conifer shoot will receive little light due to the mutual shading of other needles on the same shoot and even the same tree or others nearby. That arrangement of needles is often beneficial when sunny conditions are coupled with cold air temperatures, resulting in photo-damage and low temperature photoinhibition, as a high amount of needle tissue is shaded from intense sunlight that would otherwise cause damage to the photosynthetic apparatus (Johnson et al 2004). Under diffuse light, however, light is more evenly distributed and more surface area is illuminated, allowing for more needles to contribute to net photosynthesis despite the lower irradiances that are commonly associated with cloudy days. Diffuse light created by clouds lowers the irradiance of sunlight and the lower light intensity decreases the risk of photoinhibition, compared to needles irradiated with direct sunlight.

Epidermal lens cells have been shown to focus direct light in some plant species by up to 20 times the intensity of light incident at the leaf surface (Vogelmann et al. 1996). The role of epidermal lens cells under diffuse light conditions is more speculative, and it has been suggested that, compared to flat shiny leaves, they may aid in the capture of diffuse light for photosynthesis (Lee et al. 1990; Lee & Graham 1986). However, we found that leaves with lens cells reflected more diffuse light than leaves without lens cells

and, contrary to expectations, these leaves absorbed less diffuse light than leaves that lacked lens cells. The differences in absorption between these two types of leaves was small 2-3%, and was not likely to be responsible for the 10-15% differences in photosynthetic performance that we measured.

These experiments warrant a refinement in the way we think about the light environment of plants. From the top to the bottom of a plant canopy the changes in the light intensity, directionality, and spectral quality are extremely complex (Niinemets 2007). The effects of light intensity on photosynthesis are well understood, but changes in the directionality of light had previously been neglected. Until the community-level productivity measurements were performed, light directionality was not considered to be an important factor in photosynthesis (Press 1971). We now know that plant productivity is affected by changes in the directionality of light, and the data presented here imply that the effect of direct and diffuse light on photosynthesis changes when scaling from the leaf-level to the community.

Light absorption profiles in leaves changed with the directional quality of the incident light and when direct light intercepted the leaf from different directions. In leaves irradiated with direct light, as the angle of incidence moved away from perpendicular, light penetrated less deeply into leaf tissue. This has important implications for researchers interested in leaf display and light penetration into canopies when the sun is at low solar angles. Not only do steep leaf angles increase the interception of low-angle light, but now we know that such a leaf orientation will also increase the penetration of light into their tissues. A leaf with a steep display angle will ultimately become perpendicular to the sun late in the day. Such a leaf orientation will

decrease intense midday exposure to light and avoiding excessive heating while maximizing photosynthesis under the more favorable afternoon environmental conditions.

Although direct light penetrated into leaves further than diffuse light, the shapes of the absorption profiles were similar under these light regimes, irrespective of tissue anatomy. This is somewhat surprising because the elongate shape and optics of palisade cells has been compared to that of a waveguide which would be expected to channel direct light much further into a leaf than diffuse light. However, the difference in penetration of diffuse and direct light was only about 50 – 75 μm, and this was remarkably consistent in palisade and spongy mesophyll, tissues in which there are dramatic differences in cell shape, size and composition of intercellular air space. An explanation may be that, as light passes from the air into the epidermis, the leaf collimates the light. The refractive index of the leaf would collimate a diffuse beam and it may be that cellular anatomy aids in collimation as well. By way of illustration, a ray of light that strikes a leaf obliquely, say 30˚ (perpendicular = 90˚) and assuming the leaf's refractive index is about 1.4, would enter the leaf at an angle of 20.9˚. Also, the strength of this effect increases as the angle of incidence moves away from perpendicular. Thus, although light may arrive at the leaf surface in a diffuse state, as it moves past the leaf surface the change in refractive index will collimate the beam. The extent to which leaf tissues, such as palisade, aids the collimation process remains to be elucidated.

Here we find that leaves can respond developmentally to their growth light environment by altering cell size, shape, and the number of tissue layers. In addition, they alter their biochemistry and the distribution and quantity of absorbing pigments.

Sun and shade leaves had different distributions of chlorophyll within their tissues, particularly in *H. annuus*. Shade leaves showed a shift in pigment distribution toward the adaxial side of the leaf. In an environment rich in diffuse light, which does not penetrate as deeply into leaves, shade plants benefit by having more light absorbing pigments near the adaxial surface. In high intensity direct light environments the opposite is true.

Looking toward future research, the next logical step is to expand the scope of these studies to include a variety of leaf forms from different environments. Of particular interest would be a comparison of light absorption profiles in leaves with and without palisade tissue, a condition common in many understory fern and lily species. With anatomically uniform spongy mesophyll tissue spanning the vertical profile of the leaf, it may be that the shapes of the internal absorption profiles are controlled by chlorophyll concentration and distribution. Controlling profiles of light absorption by pigments alone may provide more plasticity than mesophyll differentiation where leaf adaptations to the environment are fixed. Measurement of the reflectance, transmittance, and absorptance of direct and diffuse light should also be expanded to include more leaf forms, as well as leaves at different stages of development. Finally, leaf-level photosynthetic measurements need to be performed in the field to determine the contrasting effects of direct and diffuse light on plants acclimated to their natural light environment.

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